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THE BACTERIAL CONTENT OF AIR IN CHILDREN'S HOSPITALS

STUDIES IN THE CHILDREN'S CLINIC AND THE CHILDREN'S CASTLE OF HELSINKI

BY

ANJA HELVE

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FROM THE CHILDREN'S CLINIC, UNIVERSITY OF HELSINKI, FROM THE CHILDREN'S CASTLE OF THE MANNERHEIM LEAGUE, HELSINKI, HEAD: PROF. ARVO YLPPÖ, M.D., AND FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI, HEAD: PROF. K. O. RENKONEN, M.D.

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MERCATORIN KIRJAPAINO

To my husband

A. PREFACE

It gives me great pleasure to express my gratitude to my esteemed and dear teacher and chief, Professor Arvo Ylppö, Head of the Children's Clinic and the Children's Castle in Helsinki, for giving me the opportunity for this scientific research work in these institutions and for much good advice throughout this investigation.

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In Helsinki, December 1949.

Anja Helve

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C. INTRODUCTION

Despite the great improvement in hospital hygiene, nosocomial infection is still a serious problem especially in children's hospitals where the resistance of patients is low. A nosocomial infection means bacteriologically the acquisition by a patient in one way or another of pathogenic micro-organisms not present in him on admission to hospital. The importance of air and dust in the spread of hospital infections is now considered conclusively proved. During the last few decades extensive scientific research has been carried out especially in Anglo-Saxon countries to discover the modes of spread of hospital infection and ways and means of preventing it. Seeing that climatic conditions play an important part in the spread of epidemics, that sanitary conditions in hospitals differ from country to country, and that severe cross-infections still occur also in Finland, especially in children's hospitals, it seemed necessary to study the role of air in this matter. It is also necessary to know the different paths by which diseases are transmitted and the conditions under which air contains the greatest number of pathogens in order that effective methods can be applied to disinfect the air.

D. REVIEW OF THE LITERATURE

Aerial spread of infection in hospitals and the different methods for preventing air-borne cross-infections have been actively studied since the last ten years of the 19th century, and the scientific literature published on the subject is so extensive that it seems enough to mention here only the most important results of this research work.

a. MODES OF SPREAD OF AIR-BORNE INFECTIONS

For many centuries epidemics have been attributed to atmospheric pollution. It is only since modern bacteriology began that it has been possible to study in greater detail the ways in which air conveys infection.

1. *Droplet infection.* Since the end of the last century transmission of infection by droplets has been considered an important source of contamination. By exposing culture plates near a coughing patient Flügge (1897) noted that bacteria were transmitted by droplets. He found that bacteria were conveyed by droplets relatively short distances and that the numbers that remained suspended in the air were few and hygienically negligible. About the same time Heymann (1899) and Laschtschenko (1899) recovered pathogenic bacteria from the atomized mouth secretions of a coughing patient. Transmission by droplets has been considered important especially in wound infections. Thus for instance Hartmann (1938) states that droplet infection is very important from the point of view of surgical asepsis but considers that bacterial contamination of wounds from the air lacks practical significance. Similarly Hirschfeld (1941) considers the number of bacteria which fall into a wound from the air relatively small, but says that many

wound infections are caused by droplets. The fact that droplets have a relatively short flight-range has been confirmed for instance by Jennison (1942) by means of stroboscopic-light photography. Even in the most recent time droplet spread has been considered an important factor as shown for instance by the statement of the Commission on Acute Respiratory Diseases (1946) in the United States that double-bunking, *i.e.* placing of beds one above another in dormitories — which lessens the frequency of close contact — reduced the incidence of respiratory disease in recruits by 50 per cent. On the other hand, Cruickshank and Wright (1947) regarded direct droplet spread rather as contact than air-borne infection and stated that it is perhaps not very important in streptococcal infections. Hare and Mackenzie (1946) and Duguid (1946) found that speaking and coughing produced only low counts of hemolytic streptococci in the anterior mouth secretions of carriers.

2. *Wells'* (1934) *theory* is that many of the droplets expelled in one way or another from the respiratory tract evaporate so rapidly that they form solid residues, so-called »droplet nuclei» which may remain air-borne for several minutes or hours. The larger droplets fall out of the air within one or two seconds. According to Wells and Stone (1934), droplet nuclei infection can remain alive in air for periods which permit its wide dissemination. Thus infection broadcast by droplet nuclei is dispersed and diluted whereas droplet infection is localised and concentrated (Wells 1934). This theory of »droplet nuclei» led Wells (1939) to the hypothesis that the semienclosed atmosphere of human habitations provides a vehicle for the epidemic spread of contagion. Wells *et al.* (1946) have stated that dust-borne infection clearly differs from that caused by droplet nuclei. According to them, infected dust particles are large and settle rapidly and therefore contaminate the air only in limited areas, causing endemic infections. Together with Ratcliffe and Crumb, Wells (1948) carried out quantitative experiments upon animals to confirm his hypothesis regarding the importance of droplet spread. These studies of quantitative air-borne tuberculosis in rabbits revealed that inhalation of bacteria in small nuclei of droplets is more dangerous, even if they are few in number, than organisms in coarse particles, because the latter are strained out in the upper respiratory tract.

3. *Dust-Borne Infection.* About the same time as droplet infection became known it was also demonstrated that the dust in rooms may contain pathogenic bacteria and that dust thus may be a vehicle for infection. Neisser (1898) noted that in some cases infective material may enter dust and survive in it for long periods causing reinfection. He did not, however, consider it probable that this dust might be reprojected into the air and so constitute a permanent source of infection. The observation that under the influence of air and light bacteria remain viable in air as droplets for only short periods of time made Kirstein (1900) conclude that dust must be responsible for cross-infections. Dust has, in fact, been regarded as the most important vehicle for aerial spread of bacteria since Lange (1928) published his extensive studies and demonstrated that dust can harbour many types of infecting agents for long periods. In addition, he was able to show that dust particles, which are smaller than droplets, may penetrate deeper into the respiratory tract.

The importance of dust in the origin of air-borne infections has been supported by the results obtained by many observers who found viable pathogenic bacteria in the dust of different inhabited rooms and hospital wards. Thus Winslow and Kligler (1912) found that streptococci are capable of surviving in dust long periods, Deicher (1928) recovered viable streptococci from the rooms of scarlet fever patients, Horewood (1931) from the dust of hospitals and households, and Glass (1941) from ward dust. Neufeld (1927) demonstrated the presence of tubercle bacilli in the dust of tuberculosis hospitals. Hart and Scheibel (1939) stated that staphylococci have been recovered in great numbers from a surgical hospital. According to Wright *et al.* (1941), *Corynebacterium diphtheriae* may survive in the dust of hospital wards in sufficient numbers to constitute a risk of cross-infection. Dold and v. Essen (1944) noted that the number of diphtheria bacilli in hospital wards was related to the severity of the disease, the number of patients, ventilation, and the atmospheric dust content. Loosli *et al.* (1943) were able to show that also the influenza virus A may survive in dust under favourable conditions.

As regards the survival rates of different bacteria in dust the results of Buchbinder *et al.* (1941) may be mentioned; according to them the virulence of dust-borne hemolytic streptococci and

pneumococci in dried state in dark is unimpaired for some weeks. Garrod (1944) also showed that streptococci may survive in naturally infected dust in dark at room temperature for a few months.

Secondary sources of infection. Several investigators, for instance Hamburger *et al.* (1944), stated that the bacteria in the dust of a room constitute a secondary reservoir from which bacteria may be reprojected into the air owing to different causes, thus spreading infection, and that the greater number of the hemolytic streptococci in the air probably originate in these secondary reservoirs, relatively few being present as droplet nuclei — as assumed by Wells. Robertson *et al.* (1944) studied the spread of streptococcal infection in army camps in the United States and noted that these secondary sources of infection, *i.e.* the dust round the bed and in the bedclothes, are of essential importance. They were able to demonstrate, both clinically and experimentally, that the number of pathogenic bacteria in the air is important in the spread of air-borne infections; this also argues in favour of the fact that dust with its high bacterial content is a more important source of infection than are droplet nuclei. In keeping with this is Loosli's (1947) statement that — though the relative importance of dust, droplet nuclei and droplets in the spread of disease of bacterial and virus etiology is not well known — it is clear that »the most important environmental reservoirs of pathogens in hospital wards are the floors and bedclothes».

b. SAMPLING OF THE AIR FOR ITS BACTERIAL CONTENT

1. *General methods.* For the purpose of quantitative determination of the bacterial content of air different devices have been constructed. Of these should be mentioned the Wells centrifuge (1933), in which the technique involves the centrifugal precipitation of measured volumes of air at set speed upon a nutrient medium, the air bacteria being deposited direct upon the medium and cultured. This is the accepted method though many investigators have later modified it. Other air samplers of different types are the »Bead-Bubbler» devised by Wheeler *et al.* (1941) and the »Atomizer-Bubbler» invented by Moulton *et al.* (1943); in both of these the technique involves the slow bubbling of a given amount of air through liquid media into which bacteria are released. Further there is Hettche

and Schwab's (1940) air sampler in which the bacteria are made to pass through glass sinter in order to be evenly distributed on the medium. Among devices in which the air passes onto the medium through a narrow slit, the most widely used are the Bourdillon Slit Sampler (Bourdillon *et al.* 1941) and the Radial-Jet Bacterial Air-Sampler invented by Luckiesh and Taylor (1946). These last investigators also introduced an Electrostatic Bacterial Air-Sampler (1946); this technique is based on the fact that positively and negatively charged bacteria will be collected on petri dishes placed on negative and positive electrodes. In Finland Hirvisalo (1933) designed an apparatus for studying the bacterial contamination of air. This apparatus consists of a row of three glass balls lined with nutrient medium; when air is sucked through the glass balls bacteria are deposited onto the medium and can thus be estimated.

2. *Indicator organisms.* As early as 1904 Gordon suggested that alfa hemolytic streptococci may provide an index of the pollution of air, because, when occurring in the air of rooms, they always originate in nasopharyngeal sources. Wells (1935) in fact stated that the alfa hemolytic streptococcus can be considered an index of air contamination analogous to the *B. coli* index of water pollution. Several investigators (Wells and Wells 1936, Pincus and Stern 1937, Buchbinder *et al.* 1938, *etc.*) consider that the number of alfa hemolytic streptococci recovered from a room indicates the degree of its occupancy.

A correlation has been observed between the beta hemolytic streptococci of the air and the incidence of streptococcal infections in the room concerned. In studying the beta hemolytic streptococci of air Buchbinder *et al.* (1938) found that they are relatively rare in normal air sampled at random and that they are usually indistinguishable from similar organisms isolated directly from disease processes. Air-borne beta hemolytic streptococci have been considered an index of streptococcal infection (Robertson *et al.* 1944) on the basis of studies of air-borne infections in U.S. Army camps during the Second World War. These showed that there was a definite association between air-borne beta hemolytic streptococci and the incidence of infections of the same type.

After serological typing of hemolytic streptococci became possible by Griffith's (1934) and Lancefield's (1933) methods, transfers of air-borne infections have been noted: streptococci of

the same type have been found in infector, infectee, and in the air or dust of the room.

3. *Animal experimentation.* Conclusive evidence for air-borne infections has been provided by making test animals under different conditions inhale contaminated air. As early as 1884, Robert Koch showed that test animals died after inhaling tubercle bacilli. Trillat and Kaneko (1921) infected animals experimentally by exposing them to atmospheres containing paratyphoid and fowl cholera bacilli. In animals whose resistance had been lowered with alcohol Stillman and Branch (1925) induced pneumonia by pneumococci suspended in the air, though transmission of pneumonia by droplets had earlier been considered improbable. Wells (1940) devised a special apparatus for studying the reaction of animals to atomised droplet nuclei. With the aid of this apparatus it was shown that pulmonary tuberculosis could be produced in animals by placing them in a chamber into which cultures of tubercle bacilli had been sprayed (Wells and Lurie 1941). Ratcliffe (1946) found that, by studying the respiratory tract of the rabbit, quantitative enumeration of the viable tubercle bacilli in nuclei-infected experimental atmosphere is possible: there was reason to assume that in his experiments all tubercles in the lungs of the rabbits had developed from single organisms. Andrewes and Glover (1941) showed that influenza virus A may pass from one ferret to another across a distance of several feet. Wells and Henle (1941) and Loosli *et al.* (1943) have produced influenza in mice by inhalation of atmospheres containing influenza virus.

4. *Studies under special test conditions.* Aerial contamination and air-borne infections have been studied by several workers under varying experimental conditions simulating room environments. Of these may be mentioned Phleps and Buchbinder's (1941) study on the settling rates of bacteria through the air and Buchbinder's (1942) study on the bactericidal effects of daylight. An article by Robertson *et al.* (1946) describing extensive researches in specially constructed experimental rooms also deserves notice. The construction of these experimental rooms makes it possible to study the effects of temperature and humidity on air-borne bacteria and viruses in relation to their survival, infectiousness for animals, and the susceptibility of bacteria to different lethal vapours. These rooms have also been used for studies of the dis-

persion into the air and distribution into the environment of respiratory micro-organisms by human subjects. In addition, they are suitable for studies of aerial infection in constantly flowing atmospheres.

C. MAN AS AN INFECTOR OF AIR

1. *Aerial pollution from human nasopharyngeal sources.* In 1905 Hamilton showed that, in addition to people suffering from streptococcal respiratory infections, also healthy people — so-called carriers of streptococci — expel these organisms into their environment. Hare (1941) demonstrated that these healthy carriers may also infect their surroundings. According to him, nasal carriers are more likely to disseminate infections than throat carriers and hemolytic streptococci identical with those found in the throat and nose of the carrier may be isolated from his skin and clothes. Alone and with his co-workers, Hamburger (1944, 1945, 1945, 1945, 1946) published results of extensive studies of streptococcus carriers in relation to spread of infection. The first few of these studies dealt with the transmission of hemolytic streptococcus infections in army hospital wards and showed that there was no close correlation between the incidence of cross-infection and the throat carrier rate; also that carriers of hemolytic streptococci whose nose cultures were strongly positive represented a very dangerous group, being responsible for the majority of hospital streptococcal cross-infections. Such a heavy carrier may release millions of hemolytic streptococci by one sneeze. Hamburger *et al.* (1949) further demonstrated that during streptococcus epidemics the ratio of nose to throat carrier rates is considerably higher than when the incidence of such infections is low.

Dumbell *et al.* (1948) stated that by gentle manipulation of a dry handkerchief as many as 50,000 bacterial particles from the respiratory tract may be distributed into the air. According to Hart and Upchurch (1942), »the degree of contamination of the front of the operating uniform of nurses, doctors, and orderlies varies directly with the nose and throat flora of the wearer and the length of time the uniform is worn». This agrees with what has been stated above.

2. *Pollution of air from other human sources.* It has long been known that infectious agents may be dispersed into the air from

infected wounds. Yet it has not been possible to study this question systematically until the introduction of bacteriological air analysis. Cruickshank (1935) found numerous streptococci in the atmosphere of burns wards and they spread into the air in particularly great numbers when the dressings handled in the ward were dry. Bourdillon and Colebrook (1946) and Miles (1940) showed that streptococci were disseminated into the air from infected war wounds. Hare and Willits (1941), likewise, demonstrated that the handling of dressings of streptococcal wounds and of bedclothes liberated considerable numbers of these organisms. According to Bourdillon and Colebrook (1946), large clouds of bacteria, such as streptococci and *Staphylococcus aureus*, were released by the movement of pyjamas or bedclothes in dressing rooms for burns. White (1936) said that large numbers of streptococci may be projected into the air from dried lochiae, by movement of dressings of patients with puerperal fever, and that these organisms may then cause cross-infection.

d. AERIAL TRANSFER OF INFECTION

Numerous instances of aerial spread of infection have been described by different investigators. An observation of Cruickshank (1935) has been quoted above, *i.e.* that greater numbers of hemolytic streptococci occurred in the burns wards than in the air of other wards of the same hospital. He was also able to show that the rapid increase in the incidence of infection of burns after admission to hospital was due to cross-infection from cases carrying or infected with these micro-organisms. White (1936) reported an interesting observation: a ward orderly cleaning a ward was infected with dust-borne streptococci of the same type as had been recovered from a patient with puerperal fever nursed there five days previously. Also, Allison and Brown (1937) found that the complications and relapses of scarlet fever patients were caused by bacteria of different serological type from that causing the primary disease, the reinfecting types of bacteria being recovered from the air of the ward. Lichtenstein's observation, as early as 1932, that complications of scarlet fever were much less common in the isolation than in the general ward, agrees with the above. Allison (1938) also noted that in measles wards the types of streptococci were

exactly those found as infecting agents in the patients. Keevil and Camps (1937) studied the spread of streptococcal infections in general wards and considered that the source of infection was usually infected dust; they exposed culture plates in these wards and showed that streptococci of the same types as the infecting agents grew on these plates. Okell and Elliott (1936), again, studied the spread of streptococcal infections in otorhinological wards and concluded that these patients run considerably more risk at hospital than they would have done if they had been nursed at home. Cruickshank and Godber (1939) consider that also puerperal fever may be transmitted by air. An instance of air-borne infection in a surgical hospital is quoted by Fingerland (1942); a doctor with catarrhal rhinitis caused a serious epidemic of streptococcic wound infection: seven patients were infected and five of them died. Cristie and Wright (1941) found virulent diphtheria bacilli in the air and floor dust of diphtheria wards at times when cross-infections with these organisms were occurring. Bøe (1946) has described an epidemic in Norway of air-borne infection in newborn infants caused by staphylococcus carriers, and Muhl (1948) has reported a similar epidemic in Sweden, also due to carriers; in most cases air-borne spread could be demonstrated. Laurell *et al.* (1947) described air-borne infections due to carriers in a children's hospital in Sweden.

e. STERILISATION OF AIR

The existence of air-borne infections is supported by the fact that various effective measures for the reduction of aerial contamination and for the suppression of dust in wards have brought about a fall in the incidence of infection. Long ago attempts have been made to control or prevent infections by destroying the infecting agents in the air. Even Lister, in his time, tried to disinfect the air and control wound infection during operations by means of his famous phenol sprays. The methods for eliminating air-borne bacteria now in use are based firstly on the inactivation of bacteria and viruses in the air by various germicidal sprays and by ultraviolet irradiation, and secondly on different dust control measures.

1. *Aerosols.* The French scientist Trillat (1938) has studied different possibilities of preventing the aerial spread of pathogens

by antiseptic sprays, so-called aerosols. The strong bactericidal effect of these aerosols is due to the fact that a very small amount of chemical substance can be dispersed as very small droplets throughout a relatively large enclosed air space. As each droplet contains the same concentration of the chemical substance as the parent solution, the bactericidal agent is enabled to act in high concentration on air-borne bacteria in a large space. Trillat tested a great number of different chemical compounds but found only two, resorcinol and sodium hypochlorite, satisfactory. Later several workers have tested the bactericidal action and toxicity of many different substances. The observation, made by Robertson *et al.* (1941), that certain glycols are very effective in the sterilisation of air, is important. According to Robertson (1943), the basis for the germicidal action of glycols is that abundant collisions occur between the hygroscopic glycol molecules and bacteria-containing droplets. Hamburger *et al.* (1945) presented evidence that triethylene glycol vapour reduced considerably the bacterial content of air, under experimental conditions as well as in hospital wards, if the humidity and triethylene concentration remained within definite limits during the test. Puck *et al.* (1943) and Acklin (1946) showed that also propylene glycol acted as a powerful bactericidal agent in a certain moisture and concentration.

Lester *et al.* (1949) studied the effectiveness of triethylene glycol vapour on micro-organisms dispersed into the air as small droplets and arrived at the result that, under optimum conditions, the relative moisture of air being 15 to 40 per cent and the saturation percentage of triethylene glycol vapour 40 to 100 at room temperature, the action is very rapid. Within the first minute about 80 to 90 per cent of the air bacteria were killed and by 3 to 4 minutes air samples were essentially sterile.

Several workers have confirmed these results, for instance the Swede Gedda (1949) who found that triethylene glycol considerably reduced the bacterial content of air in the waiting room of an oto-rhinological ward and also in a children's home.

In a crowded room Challinor and Duguid (1944) obtained about 85 per cent reduction in the naturally present air contamination by means of propylene glycol vaporisation and concluded that bacteria dispersed in droplets are more susceptible than dust-borne bacteria to the action of glycol.

Propylene and triethylene glycol have also been observed to lower the incidence of infection, for instance by Harris and Stokes (1945) in a children's ward for chronic cases. On the other hand, Loosli and Smith (1949) did not find that infections were significantly reduced by means of triethylene vapour in a children's hospital.

2. *Dust-control measures.* Attempts have been made long ago to prevent the distribution of dust into the air from floors by treating floors with various dust-laying substances. As early as 1909, the German Heise published a study on the treating of floors with different oils for reducing the amount of dust liberated in printing works. That the amount of bacteria redispersed into the air in hospital wards can be reduced by treating the floors with spindle oil has been demonstrated by van den Ende *et al.* (1940) and by Thomas and van den Ende (1941). Van den Ende *et al.* (1941) were able to show that oiling of bedclothes reduced the amount of bacteria released from infected bedclothes during bed-making by as much as 90 per cent and van den Ende and Thomas (1941) reported 99 per cent reduction by the use of oil-in-water emulsions. Such dust-laying measures have also been shown to reduce the incidence of infections. Water-soluble triton oil, used by Robertson (1943) in Chicago for impregnation of bedclothes, proved very effective. Anderson *et al.* (1944) showed that oiled floors were effective in controlling respiratory infections, and Wright *et al.* (1944) that, in a measles ward where all bedclothes, ward-linen, and floors were oiled, the streptococcal cross-infection rate was lower than in the control ward. On the other hand, the Commission on Acute Respiratory Diseases (1946) found, on the basis of a study of new recruits in the U.S.A., that oiling of floors and bedding reduced the incidence of hospitalised respiratory disease during a period of low endemic occurrence, but that it was of little or no effect during epidemics of acute respiratory infection. Laurell (1948) reports that oiling of floors and bedclothes did not reduce the number of secondary infections in a children's hospital in Sweden; there occurred even more infections in the oiled ward.

3. *Ultraviolet irradiation for sterilisation of air.* Downes and Blunt, in 1878, observed that ultraviolet light destroyed bacteria. Wells and Wells (1936 and 1938) and Wells (1935) applied this to

air-borne bacteria and hospital conditions in the control of aerial spread of bacteria and infection. On the basis of the good results obtained, Wells and Wells (1942) concluded that since the dynamic spread of epidemic contagion can be controlled by radiant disinfection of air, it follows that air must be a vehicle of infection. Several other workers have later used ultraviolet irradiation and been able to confirm the above observation regarding the bacterial content of air: they also noted that it lowered the incidence of infections. For instance Mc Khann *et al.* (1938) reported a distinct reduction of the number of air-borne cross-infections in irradiated children's and infants' wards. Rosenstern (1942) noticed that infections decreased in number in children's homes after introduction of ultraviolet radiation. In addition, del Mundo and Mc Khann (1941) described a reduction of acute respiratory diseases from 12.5 to 2.7 per cent in a children's hospital, and according to Hart (1942) the infection rate in clean wounds in an operating room was strikingly reduced. Greene *et al.* (1941) obtained good results by ultraviolet disinfection of air in preventing epidemics of varicella, and M. Wells (1945) considers it an effective means of preventing the spread of chickenpox and measles in class rooms. It is of interest, further, that according to C. E. Robertson *et al.* (1939), premature infants nursed in irradiated wards showed nearly 50 per cent less infections than those nursed in ordinary premature wards. Moreover, Wheeler *et al.* (1945) stated that respiratory infections in U.S. Naval Training Centres were reduced by 30 to 40 per cent by sterilisation of the air.

Although numerous good results have been obtained by ultraviolet radiation in the reduction of air-borne infections, Mitman (1945) has, on the other hand, stated that its chief limitations are its ineffectiveness in the presence of dust and its poor penetrating power. According to Recent Studies on Disinfection of Air in Military Establishments, published in 1947 in the U.S.A., ultraviolet irradiation is ineffective in the case of dust-borne bacteria and viruses. In addition to radiation some aerosol is therefore required in order that good results may be obtained.

E. OBJECTS OF THE INVESTIGATION

The present study aims at discovering the extent to which bacterial infections are transmitted by air in a children's hospital in Finland and the degree of aerial pollution in its wards. An attempt is made to give a picture of the general bacterial content of the wards, by observing the total number of colonies, alfa hemolytic streptococci as a index of air pollution, beta hemolytic streptococci as an index of streptococcal infections, and *Staphylococcus aureus;* all this with due reservations for the limitations of technical equipment, etc. The plan also included a rough study of the relative importance of the different groups of bacteria in the total bacterial content. These results have been compared with those obtained abroad with a view to discovering to what extent sanitary conditions in Finnish hospitals differ from corresponding conditions in other countries as regards air contamination.

The study further aims at clarifying the relative importance of the different types of diseases in the bacterial content of the air. Attention has been given to the influence of seasons, the humidity of ward air, and to the nursing and cleaning activities at different times of the day. Features connected with the hospital lay-out and architecture were also examined as to their share in aerial transmission of infections. Finally the possible importance of air sterilisation as a means of controlling hospital infections was studied. It must be remembered, however, that these investigations did not include viruses.

Throat and nose swabs were taken from members of the staff to determine the carriers of streptococci and staphylococci because it is well known that transfer of infection may occur also by healthy carriers. For comparison air samples were collected from the Children's Castle (See p. 72) on completion of the building and

after admission of patients, for the purpose of studying how their presence affected the bacterial counts. The question whether home or hospital environment is in general safer for children from the point of view of aerial contamination has been illustrated by a few examples: samples were taken from rooms in households of different social status in which there were children previously nursed at the same children's hospital.

In connection with the questions described above samples were also taken of different hospital fixtures and articles to reveal their importance as vehicles of cross-infection.

Though these questions have been widely studied in several countries, it is not known that any investigations into the bacterial content of the air in hospitals have been undertaken in Finland. Because of this a systematic study of the subject has seemed necessary.

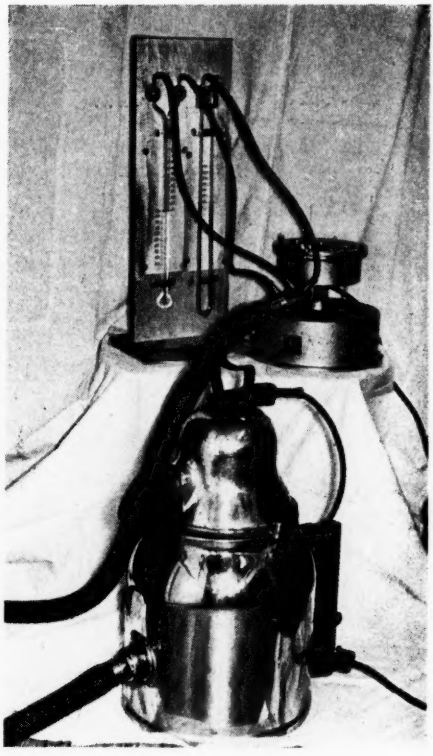
F. METHOD

The sampler. The sampler used in these investigations has been designed under the supervision of the author on the basis of the Slit-Sampler devised by Bourdillon *et al.* (1941), said by them to give a collection efficiency of 96 per cent, and the Radial-Jet-Sampler devised by Luckiesh *et al.* (1946), said by them to collect over 95 per cent of air-borne bacteria.

The apparatus, shown in Fig. 1, has three parts: a sampler proper of metal, a venturimeter, and a vacuum motor for suction. (It has been made by Messrs. Santasalo-Sohlberg Oy.) The sampler proper (shown graphically in Fig. 2) is a cylindrical sealed chamber, inner diameter 130 mm. and height 42 mm., with an air-tight lid secured by rubber packing and 4 thumb screws. In the middle of the lid is a round opening, 26 mm. in diameter, through which air is sucked in; on the sides are round glass windows. The inlet is connected by a screw mechanism to a low cylindrical metal box within the sealed chamber; the box is 20 mm. high and in its bottom, detachable by means of a screw, is a radial slit, 35 mm. long and 0.2 mm. wide. In the sealed chamber is a revolving metal platform, 80 mm. in diameter, on which the culture plate rests. At the edges of the platform are 3 graduated vertical rods to keep the culture plate in place. The platform is detachable and its centre is fastened to the axis of the driving motor under the sealed chamber; the platform revolves round this axis. At the side of the driving motor is a regulator to control the rate of the culture plate's movement. In the bottom of the sealed chamber is the outlet for air; its diameter is 25 mm. and the metal sleeve of the venturimeter may be attached to it. This metal sleeve has three openings of which two are attached by rubber tubes to the water manometer of the venturimeter and the third to a mercury manometer. The other end of the metal sleeve of the venturimeter is fastened by thick rubber tubing to the vacuum motor which has an adjustable rheostat.

The apparatus includes an auxiliary device — the base seen in Fig. 3 — on which the covered culture plate, resting on its platform, may be placed; this is used when the distance between the slit and the culture plate is being adjusted. (This corresponds in Bourdillon's sampler to a screw

Fig. 1.
THE APPARATUS USED

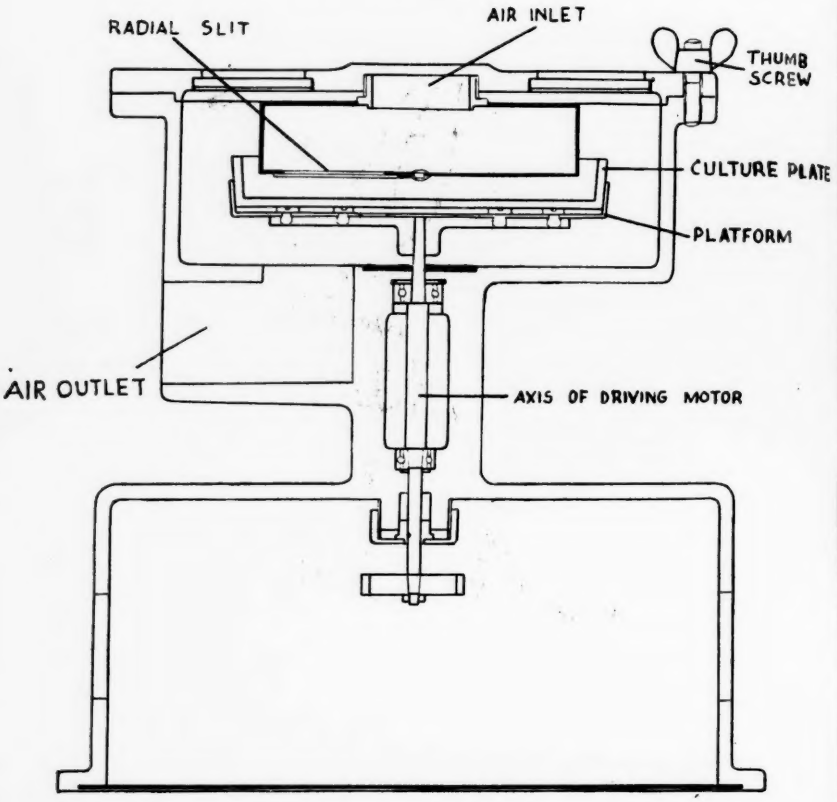


mechanism by which the axis of the revolving platform may be shortened or lengthened.) By placing metal disks, 0.2 mm. in thickness, under the bottom of the culture plate it is possible, with the aid of the graduated vertical rods, to make the distance between the slit and the culture surface 2 mm. This is considered to be the most suitable distance (Bourdillon *et al.* 1941) for a maximal number of bacteria to impinge on the culture surface.

When the required 2 mm. distance between the slit and the culture surface has been obtained, an uncovered culture plate is placed in the sealed chamber which is closed with the thumb screws. The volume of air drawn in per minute can be read off the scale of the venturimeter, and the mercury manometer shows the difference between the atmospheric pressure in the room and that of the venturimeter. The efficiency of the suction motor and the rate of flow of the sucked-in air may be regulated by means of the rheostat. The revolutions of the plate may be followed through the window in the chamber, and with the driving motor the rate

Fig 2.

DIAGRAMMATIC DRAWING OF SAMPLER

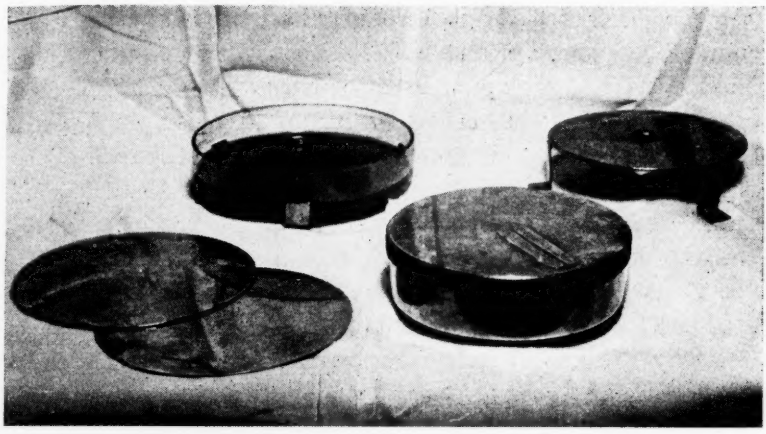


may be fixed at 2 revolutions per minute which has been found to be most advantageous (Bourdillon *et al.* 1941). These authors also showed that sterilisation of the infection chamber does not essentially affect the results. The chamber and the slit of the apparatus used in these investigations were wiped daily before sampling with a piece of cotton immersed in alcohol (96 per cent).

By such a suction method it is not possible to determine the absolute number of bacteria present in a given volume of ward air, because more than one organism may cling to the same dust particle and part of the colonies growing on the culture plate may originate from more than one organism (du Buy *et al.* 1945, Luckiesh *et al.* 1946). Moreover, only those bacteria which have

Fig. 3.

ACCESSORIES USED FOR ADJUSTING THE DISTANCE BETWEEN THE SLIT AND THE CULTURE PLATE SURFACE



grown into visible colonies are counted. Garrod (1935) has shown that single bacteria take considerably longer than clusters of bacteria to grow to a visible colony.

During sampling the conditions in the ward vary and the bacterial content of the air is constantly affected by such factors as air currents, people in the ward, *etc.* Even such slight activity as the changing of a culture plate in the sampler by the observer may considerably increase the bacterial content of the air because bacteria are liberated from the observer's skin and clothes (Duguid and Wallace 1948).

METHOD OF SAMPLING

Samples of ward air

For the total bacterial count air samples were collected on 10 per cent human blood agar plates exposed to 100 liters air. This corresponds to a 3 min. run, the rate of the sampler being 33 to 33.5 liters of air per min. This volume of air was chosen in order that the results might be comparable with those obtained in Scandinavia where 100 liters has also been used in determining the total colony count (Laurell *et al.* 1947, Löfström 1947, *etc.*). After sampling the plates were incubated at 37° C. for 24 hours. If growth on these plates was so heavy that visual counting of colonies was

difficult, these were counted with an electrical counter (constructed in the Children's Clinic with the aid of the Telephone Company).

The growing colonies were classified as follows: the larger colonies were stained directly by the Gram method, the small colonies first cultured on 10 per cent human blood agar plates and then stained. Staphylococci of the aureus group were classified from the blood agar plates after 24 hours at 37° C. as described above and an additional 24 hours at room temperature by observing their pigment formation (Packalén and Bergqvist 1947).

Classification of alfa and beta hemolytic streptococci. Garrod (1933) has stated that gentian violet is a selective medium for streptococci and Thomas and van den Ende (1941) that, used in a concentration 1: 500,000, it reduces the growth of aerial saprophytes by 95 per cent without seriously inhibiting pathogenic streptococci. Schneiter *et al.* (1947) have also dealt with the selection of culture media for air sampling and found that gentian violet is a suitable selective medium for aerial streptococci.

In these investigations a gentian violet culture medium modified by Kokko (1946) was used in culturing and identifying airborne streptococci. The composition and preparation of this culture medium is as follows:

Bacto-Tryptose (Difco)	20.0
Yeast extract (Difco)	5.0
NaCl	8.0
Glucose	0.5
Phosphate buffer solution, pH 7.6 (KH_2PO_4 1.45, $Na_2HPO_4 \times 2H_2O$ 7.60, Distilled water ad 100.0)	22.0
Bacto-Agar (Difco)	12.0
Distilled water ad	1000.0

All the above substances were mixed and autoclaved for 15 minutes. Then the pH of the mixture was adjusted to 7.4—7.6 with 10 per cent sodium bicarbonate solution, using Beckmann's pH-meter. After this the mixture was again autoclaved for 10 min., filtered and measured into flasks. These were sterilised for 15 min. at 120° C. Immediately before the agar mixture was poured on the plates, 5 per cent horse blood and an aqueous solution of gentian violet were added to make up 1: 1,000,000.

After sampling, the plates were incubated for 48 hours at 37° C. and the colonies immediately counted.

To identify Staphylococcus aureus bacto-phenol red mannitol agar, with an addition of salt as recommended by Chapman (1945), was used. Evans (1948) studied coagulase-positive types of staphylococci and found that this was a satisfactory selective medium, and Laurell *et al.* (1949) used it for isolating air-borne staphylococci of the aureus group. The composition and preparation of the medium was as follows:

Proteose Peptone No. 3 (Difco)	10.0
Bacto Beaf Extract (Difco)	1.0
NaCl	5.0
Agar-aga	15.0
Distilled water ad	1000.0

The above mixture was allowed to stand at room temperature for 2 hours and then autoclaved for 30 min. and strained through sterilised gauze and cotton. To this was added 12.5 cc. of a 0.2 per cent phenol red solution. The pH was adjusted with 10 per cent sodium bicarbonate solution to 7.8, using Beckmann's pH-meter. After adding 70.0 NaCl and 5.0 mannite the mixture was autoclaved for 15 min., bottled and sterilised at 120° C. for 20 min.

After sampling the plates were cultured at 37° C. for 24 hours and at room temperature for another 24 hours, and the colonies immediately counted.

For classification of streptococci and *Staphylococcus aureus* 20-min. runs, corresponding to 666.7 liters of air, were made. Tests with runs of varying length (5 to 30 min.) using these special culture media were made. It was found that 20 min. was the most satisfactory time of sampling considering the collection efficiency of the apparatus and the density of colonies on the plates.

Settling plates

The simplest and oldest method for determining the bacterial content of air is by exposure of settling plates in a room for definite periods of time. By this method, however, the bacterial content of a known volume of air cannot be studied, also only the coarser particles will settle on these plates (Wells and Riley 1937, Crumb and Wells 1947).

For the settling plates the two above described selective media, gentian violet agar and Chapman agar, were used. To prevent drying of the medium it was poured on the plate about 0.8 to 1 cm. deep and the uncovered plates were exposed for 8 hours at a height of 30 cm. from the floor in the middle of the room studied. The plates were cultured as related above.

Samples from sources other than air

Throat and nose swabs were taken in the usual way on a piece of cotton, planted directly on 10 per cent human blood agar plates, and incubated for 24 hours at 37° C.

From water taps, door handles, baths and nail-brushes samples were taken either on a dry sterile cotton swab or on a cotton swab immersed in sterile broth, then planted on 10 per cent human blood agar, and cultured as described above.

Freshly laundered ward linen, for instance diapers, bibs and knickers, was sampled as follows: The article of clothing was placed on a table over a sterile petri dish, care being taken to touch only the edges of the article. Sterilised glass beads (1 hour at 180° C.) were then poured over the plate covered by the article and 20 cc. of sterile broth was pipetted onto the beads. The plate was covered and shaken for 3 min. After this the article, with the beads, was lifted from the dish and the broth remaining on the dish was incubated for 24 hours at 37° C. and planted on 10 per cent human blood agar. If broth had been absorbed through the clothing onto the table the sample was considered a failure and not cultured. This method was chosen because it was necessary to use as small amounts as possible of culture medium.

Assmann's ventilation psychrometer was used for determining *the relative humidity of air,* and this was done at the same time and in the same place as the total bacterial content was determined.

G. MATERIAL FROM THE CHILDREN'S CLINIC

The material studied for these investigations is from the new Children's Clinic in Helsinki, opened to patients in the late summer 1946 and having 270 beds for children of different ages. The regular staff of the hospital numbers 305.

With a view to preventing the spread of infection the hospital building (Ylppö 1948) is designed so as to contain in its curved central part the common X-ray department, the laboratory, and the administrative section. From this central part the units extend like the fingers of a hand. In its highest part the building is four-storied. Each of the 13 units has single and double wards for purposes of segregation, and the largest wards are for 6 children. To control the spread of infection the hospital has a special isolation unit for such children whose disease is doubtful or who may be considered to harbour infection, and in addition, a special epidemic unit especially for children developing infectious diseases in the other units of the hospital.

There is a forced ventilation plant which sucks out the vitiated air from each ward separately; the used air is replaced by warm fresh air which first enters the central corridor and from there under positive pressure the patients' and other rooms. The premature ward has a special air-conditioning system: fresh air is brought into each ward separately. — The hospital has one central heating system.

The samples were collected in the Children's Clinic during the period Jan. 7, 1948, to Sept. 12, 1949. No special dust-laying or aerial disinfection measures were then used in those wards in which the samples were taken. The wards and corridors were swept and dusted with a wet cloth. Cleaning and natural ventila-

tion took place regularly three times a day, the latter, of course, varying with the outside temperature. — In the premature, infants', isolation and epidemic wards the staff always used protecting masks. In the premature, isolation and epidemic wards a special gown had to be used by the staff when attending to each patient; in the other units a gown for the staff hung in each ward. Hands were scrubbed before and after attending to each patient in all units. — No visitors were admitted to the premature units. In the other units visitors were admitted twice a week for one hour but not allowed to enter the wards, only to look at the children through the glass windows in the corridors. No visits of children were allowed. — The health of the staff was constantly followed and anyone showing signs of infection was not allowed on duty.

The air samples were always collected in the middle of the ward, 62 cm. from the floor. Apart from the serial samplings at hourly intervals during 24 hours, samples were taken between 9.30 a.m. and 12.30 p.m. because at this time aerial pollution is generally considered to be high and because there is then relatively little activity.

The air samplings were generally made in the same order, *i.e.* first for the total bacterial content, then for air-borne streptococci, and finally for staphylococci. During sampling any movement in the ward, except for the changing of culture plates, was avoided as far as possible.

H. PRELIMINARY MATERIAL

In order to determine the extent to which successive samples collected from the same room usually differ as regards bacterial content, two successive samplings were made 30 times in a laboratory of 38 cu.m. capacity. Stable conditions were maintained as far as possible in this laboratory. Statistical correlation of the counts thus obtained showed that the correlation coefficient of the two series was + 0.875, thus strongly positive; this showed that the successive counts were of the same order of magnitude, and the difference between these two series is not significant. In Fig. 4 (p. 34) the successive samplings are presented graphically by dots.

Preliminary samplings were made to reveal whether differences in the bacterial content of wards of different type can be demonstrated by the method used; between Feb. 2 and Sept. 15, 1948, samples were collected once from all occupied wards and in some cases sampling was repeated. At the same time settling plates were exposed in the wards. These samples showed that, even in wards of the same type, differences appeared in the total bacterial content of air as well as in the *Staphylococcus aureus* count. But a comparison of wards of different types revealed striking dissimilarities, and on the basis of these, certain wards were selected for the investigations described below.

These preliminary studies also showed that the number of streptococci, determined by air sampler and settling plates, varied more in the wards of different type than the total bacterial content and the number of staphylococci. Another observation, which has been previously reported (Helve *et al.* 1950), was that the wards in which eczematous children were nursed usually contained large numbers of air-borne beta hemolytic streptococci. These

Fig. 4.

PRELIMINARY SAMPLES FROM LABORATORY

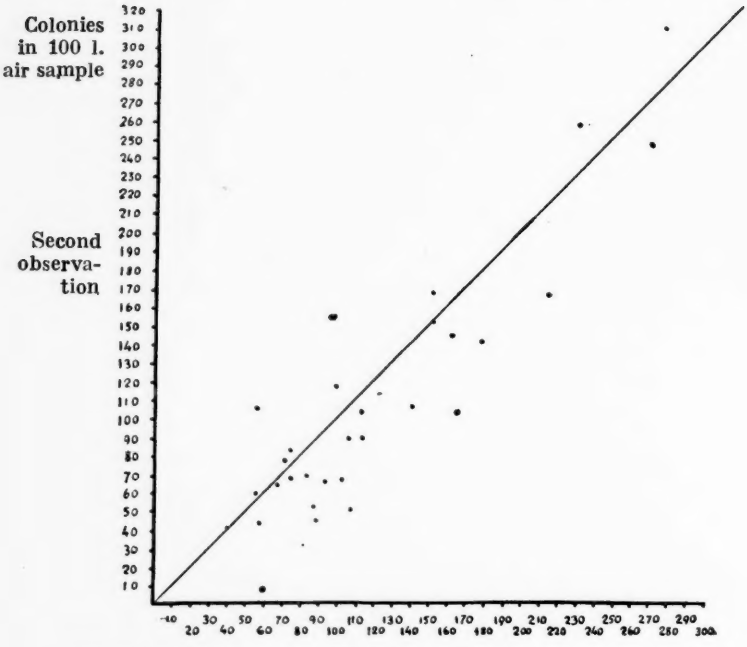


TABLE 1

BETA HEMOLYTIC STREPTOCOCCI IN THE PRELIMINARY MATERIAL

	Bourdillon Technique		Settling plate technique	
	Number of samples	Ratio of β-hemolytic streptococci to total bacterial count	Number of samples	β-hemolytic streptococcus count
Air samples from the wards housing eczematous children	11	1: 17.5	16	8
Samples from other wards	105	1: 403	62	1.0

wards were studied in greater detail (Table 1): a total of 16 samples were collected by exposing gentian-agar settling plates for 8 hours. The number of beta hemolytic streptococci averaged 8 per plate, whereas in other wards, in which 62 settling plates were exposed for 8 hours, their average number was 1.0 per plate. In samples collected with the air sampler from 11 wards for eczematous children the ratio of beta hemolytic streptococci to the total bacterial content was 1: 17.5 but in 105 samples collected from other wards it was 1: 403. Accordingly a ward for eczematous children was selected for systematic study.

I. THE MATERIAL PROPER

General investigation procedure

The material proper comprises samples collected 20 times at each of the different observation posts in the hospital during one year, or from Sept. 20, 1948, to Sept. 12, 1949. The samples were taken at as regular intervals as possible, always on three successive days, the order of the wards being invariably the same. The sampling was always done as follows: first a 3-min. run for the total bacterial content, then a 20-min. run for the streptococcus count, and finally a 20-min. run for *Staphylococcus aureus*. The observation posts were as follows.

1. A three-bed ward in the older children's unit. The volume of air per child was here on an average 19.5. cu.m. Most of the children nursed in this ward during the period of the present study had various rheumatic diseases, renal disease, diabetes, or other affections of chronic type.

2. A double ward in the premature infants' unit. The volume of air per infant averaged 27 cu.m. The premature infants nursed here were usually free from infections, except for 2 cases of otitis media and 2 of pneumonia. The infants were not kept in incubators.

3. The next ward, a double premature ward. The volume of air averaged 26 cu.m. per infant. No incubator was used and the infants were mostly free of infections, except for 2 cases of pemphigus neonatorum and one infant who had peritonitis metastatica as a result of septic omphalitis.

4. A double infants' ward housing chiefly patients with gastrointestinal disturbances. The volume of air per infant was on an average 24 cu.m.

5. The next unit, also a double ward. In this ward, too, chiefly infants with gastrointestinal disturbances were nursed during the period studied. The average volume of air per infant was 22 cu.m.

6. A four-bed ward in the surgical department with an average air volume of 16 cu.m. per child. In this ward the patients were older children with different surgical diseases.

7. A single-bed cubicle in the isolation unit. The walls of the cubicle were 2.15 m. high; its area was 4 sq.m. and that of the ward from which it was partitioned off was 37 sq.m. The total cubic content of the ward was 110 cu.m. The patients in this cubicle were chiefly acute infectious cases, for instance pneumonia, pharyngitis, paratyphoid, *etc.*

8. A three-bed ward for eczematous children. The average volume of air was 18.5 cu.m. per child. The patients were mostly children with eczema infantum, Besnier's prurigo or other skin diseases.

9. Corridor in the eczematous children's unit.

10. Nurses' room in the eczematous children's unit.

These observation posts were chosen on the basis of the preliminary samplings for the purpose of getting a picture, as consistent as possible, of the total bacterial content of the whole hospital. As already stated, control samples were collected only from the premature and infants' units because of the limited supply of culture medium and the efficiency limitations of the apparatus. These units were more closely observed because it is well known that serious cross-infections occur there. The averages of the counts obtained from these two units are given in the results. The corridor and nurses' room in the eczematous children's unit were chosen because of the interest in the extent of aerial transmission of bacteria in wards where a high bacterial content could be expected.

DIFFERENT TYPES OF BACTERIA RECOVERED FROM WARD AIR

Several workers have found that the great majority of bacteria occurring in the air of hospitals are harmless saprophytes. Thus Allison and Brown (1937) noted that the greater part of colonies cultured from air samples collected in scarlet fever wards were staphylococci of the albus type while Hart (1942) demonstrated

that over 90 per cent of the organisms recovered from the air of an operating room were staphylococci. The same observation was made in a children's hospital by del Mundo and McKhann (1941) who added that they very seldom recovered pneumococci. According to Hamburger *et al.* (1944), most of the bacteria in the air of inhabited places are saprophytes, for instance clumped gram-positive cocci, *B. subtilis* and molds, and the flora is similar to that of the floor dust. Buchbinder *et al.* (1945) also studied air-borne bacteria of enclosed spaces and found that these are chiefly saprohytes, and also those expelled by the inhabitants. Colebrook (1946), who studied the bacterial content of air in burns wards, found that the majority of organisms were harmless types, *i.e.* diplococci and diphtheroid bacilli, very often *Staphylococcus aureus*, more rarely *B. pyocyaneus*, coliform bacilli, hemolytic streptococci and *B. proteus*.

As it seemed of interest to study the extent to which different types of bacteria occur in ward air in Finland, all colonies grown on 121 petri dishes from 100 l. air samples collected in the different parts of the hospital have been analysed by the aid of Gram's staining method and microscopic examination. It appeared that here, too, the greater part were saprophytes (*Staphylococcus albus*, *Sarcinae*, and large gram-positive cocci). Colonies of different types of bacteria occurred in these samples as follows (average):

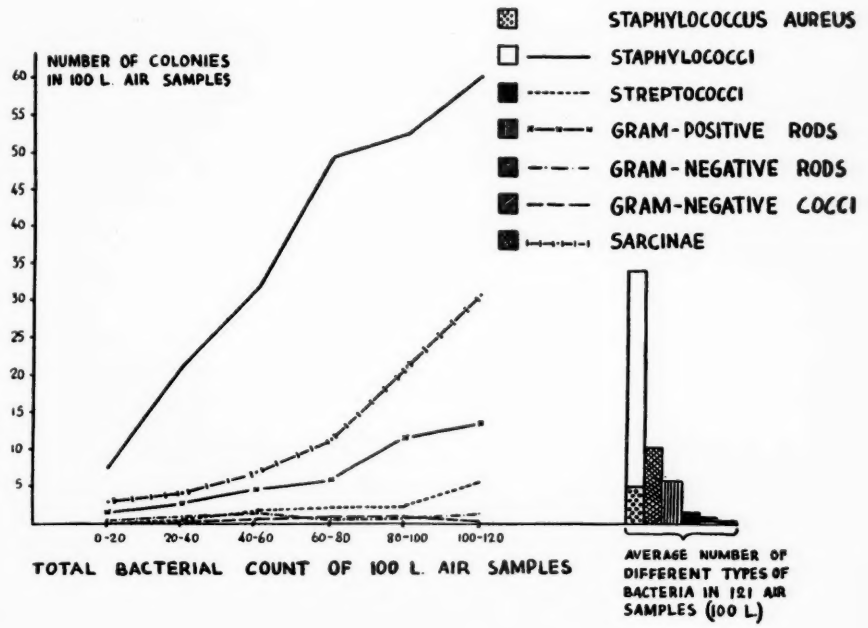
Staphylococcus albus	28.9	33.7
Staphylococcus aureus	4.8	
Alfa hemolytic streptococci	0.3	
Beta hemolytic streptococci	0.4	1.8
Non-hemolytic streptococci	1.1	
Gram-positive rods	5.8	
Gram-negative rods	1.2	
Gram-negative cocci	0.9	
Sarcinae and large gram-positive cocci	10.3	

The proportion of gram-negative bacteria was thus usually small compared with the gram-positive.

Fig. 5 shows the changes taking place in the number of different types of bacteria as the total bacterial count increases. The diagrams indicate that the number of gram-negative rods and cocci

Fig. 5.

DIFFERENT TYPES OF BACTERIA IN RELATION TO TOTAL COLONY COUNT



did not increase with the total bacterial content, but that of gram-positive bacteria did increase proportionately. As it has earlier been observed that gram-positive cocci and rods in dried state remain viable longer than for instance gram-negative rods of the coli group (Wells 1936), this would argue in favour of the fact that, at least in the presence of considerable bacterial contamination of the air, the bacteria would mostly originate in the dust of the room, *i.e.* in the so-called secondary reservoirs (Hamburger *et al.* 1944, Robertson *et al.* 1944).

The picture thus obtained of the bacterial flora of the Children's Clinic is to some extent incomplete because, in the analyses of colonies, it was for technical reasons possible to use only the plates having a culture density of 120 or less colonies per plate; therefore about 20 per cent of the plates had to be disregarded.

RELATIONSHIP OF AIR-BORNE BACTERIA TO CROSS-INFECTIONS OF DIFFERENT TYPES IN CHILDREN'S HOSPITALS

The most usual nosocomial infections in children's hospitals, in addition to epidemics proper, are respiratory, gastrointestinal and cutaneous infections.

The role of various diseases in aerial contamination has been most widely studied with regard to the dispersal of hemolytic streptococci by patients with different respiratory tract infections: it is well known, as pointed out by Robertson *et al.* (1944), that streptococcal infections of the upper respiratory tract are among the most usual and most important diseases known to be transmitted by air. In fact, as observed by Brennemann (1932) and Meyer (1913), Lichtenstein (1935), McKhann *et al.* (1938), J. Wright (1940), *etc.*, the predominant infections in infants' wards are the acute respiratory infections. That most of the air-borne respiratory infections in children's hospitals are due to hemolytic streptococci is shown for instance in the report published by Allison *et al.* (1946) on cross-infections in children's wards. It has been demonstrated that in a children's hospital in Boston one-fifth of the patients admitted acquired one or more respiratory pathogens during their hospital stay, despite the fact that the number of patients showing clinically recognisable signs of respiratory infections remained relatively low (Brooks *et al.* 1942).

It is also well known that staphylococci may cause nosocomial nose and throat infections in children, and for example Löfström (1947) considers them especially important in the newborn.

Uncertainty still prevails regarding the modes of spread of gastrointestinal infections in children and no specific indicator organism has been isolated (Crowley *et al.* 1941, Watkins and Lewis Faning 1948). Several workers, for instance McKhann *et al.* (1938) and Larsson (1945), have stated — what is now well known — that respiratory infections may assume gastrointestinal symptoms. Even if in children a gastrointestinal infection may be caused by a case of streptococcal infection, it has in most cases not been possible to demonstrate streptococci in the infectee (Allison *et al.* 1946). Many circumstances, in fact, indicate that, as postulated for instance by Nyman (1947), diarrhoea in infants is primarily caused by a virus.

As regards different eczemas of children, these have been found both to contaminate the air and spread by air. Löfström (1947) says that persons with impetigo or erysipelas are especially important infectors of air, and this agrees well with the observations on the infection of burns described above (Cruickshank 1935, Colebrook 1946). In studying cross-infections in children's wards and their control, Loosli *et al.* (1949) also found that hemolytic streptococcal infection was often caused by a patient with exudative cutaneous infection.

J. EVALUATION OF THE RESULTS

The number of bacteria in the air samples collected from the ten observation posts in different parts of the hospital during the period in question differ considerably although on each occasion the samples from the same ward were as far as possible taken at the same time of day. This is only natural as the aerial contamination in an inhabited room is very sensitive to varying physical factors. Some of the observed values are exceptionally high, evidently owing to incidental movement during sampling or to the presence of larger aggregations of bacteria in the sample, *etc.* A few exceptionally high values may considerably affect the arithmetic mean and the standard error of a series of observations, and so give a misleading picture of the particular series. For this reason the median and the semi-interquartile range are here, as sample characteristics, substituted for the mean and the standard error, and in addition the mean is given.

To determine the median and the semi-interquartile range the observed values are arranged in order from lowest to highest and divided into four groups, each containing an equal number of observations. If the number of values in a series is odd, the median q_2 is the middlemost value of the group, and, if the number of observations is even, the median is the arithmetic mean of the middlemost values of the group. The median thus shows the average magnitude of the observed values. The dispersion of the observed values on either side of the median can be measured with the aid of the so-called semi-interquartile range: this is $\frac{q_3-q_1}{2}$, in which q_1 is the middlemost value of the first observation of the arranged series and q_2, and q_3 is the middlemost value of q_2 and the last observation. By giving the median and the semi-interquartile range of

the series of observed values, it is possible to exclude from the results the first quarter, containing the lowest values of the series, and the fourth quarter containing the highest values, which particularly in this material may be exceptionally high.

1. TOTAL BACTERIAL CONTENT OF WARD AIR

As is well known and will be shown also below, the total bacterial content of ward air depends greatly upon different physical factors and varies considerably at different times of the day.

TABLE 2

CONTAMINATION OF WARD AIR ACCORDING TO VARIOUS OBSERVERS

Observer	Year	Place of Sampling	Mean Total Colony Count per 100 cu.ft. Air Samples
Chapple and Kenney	1939	Pediatric Ward	1046
Wells, Wells and Mudd	1939	Infants' and Children's Wards	496
		Premature Ward, separately air-conditioned	20
		Children's Out-Clinics	728
Mc Donald	1940	Operating and Delivery Rooms throughout the actual operating day	230.5
Long, Mc Khann and Cheney	1940	Premature Infants' Ward	20.7
		Infants' Ward	782.3
		Children's Medical Ward	958.5
Del Mundo and Mc Khann	1941	Nursery of Premature Infants	15—20
		Children's Medical Ward	350—500
		Medical Outpatient Depart.	600—700
Rosenstern	1942	Cubicle in Adoption Nursery	51.8
		Corridor in Adopt. Nursery	64.8
Wheeler and Duckett Jones	1942	Infants' Nursery	180
		Wards in Rheumatic Fever Hospital	98—186
Bourdillon and Colebrook	1946	Hospital Wards during normal activity	100 to 1000
Luckiesh and Taylor	1947	Surgical Room	130
		Surgical Room vacant	150

Therefore these factors must also be considered when comparing the total bacterial counts of the wards. Colebrook's (1946) bacteriographs illustrate the degree of variation in the total bacterial content of ward air: these were obtained by exposing blood agar plates in a Slit-Sampler during a very slow run and showed very clearly how the removal of bandages from burns increases the bacterial contamination of the room from a few bacteria per cu.ft. to an extremely dense bacterial scatter. The appended table (Table 2) shows examples of the total bacterial content of different hospital wards as recorded by some investigators. The figures given by the different investigators differ to some extent but are generally of the same order of magnitude in samples from wards of the same type. In general the bacterial density is considerably greater in older children's wards and in outpatient departments than in premature wards. The findings of Brooks *et al.* (1942) agree with this: they studied cross-infections in an infants' hospital in Boston and found that in the open wards the total bacterial counts were significantly high as compared with those in the premature wards, where in addition to air conditioning and restriction of personnel, all babies were free of respiratory infection.

The total bacterial counts of the samples collected by the author

The graphs in Fig. 6 show the median of the total bacterial count as recorded at the different observation posts, and Table 3 the median, the semi-interquartile range, and the mean. As will be seen from the figure and the table, the lowest median, 53.5, was obtained in the premature wards. The corresponding semi-interquartile range was 25 and the mean 87. The latter is considerably higher than the median and this seems to be due to the fact that the samples included two exceptionally high (360 and 436) values. These extremely high counts can no doubt be accounted for by the incidental factors described above, as no infections were present in the infants at the time of sampling which might have affected the results.

The second lowest median, 61, and the corresponding semi-interquartile range, 33, were obtained in the infants' wards housing patients with gastrointestinal disturbances. The results agree with those obtained by previous investigators as regards the aerial contamination in the wards of the youngest children (premature

Fig. 6.

MEDIAN OF BACTERIAL COUNTS IN EACH DIFFERENT TYPE OF WARD

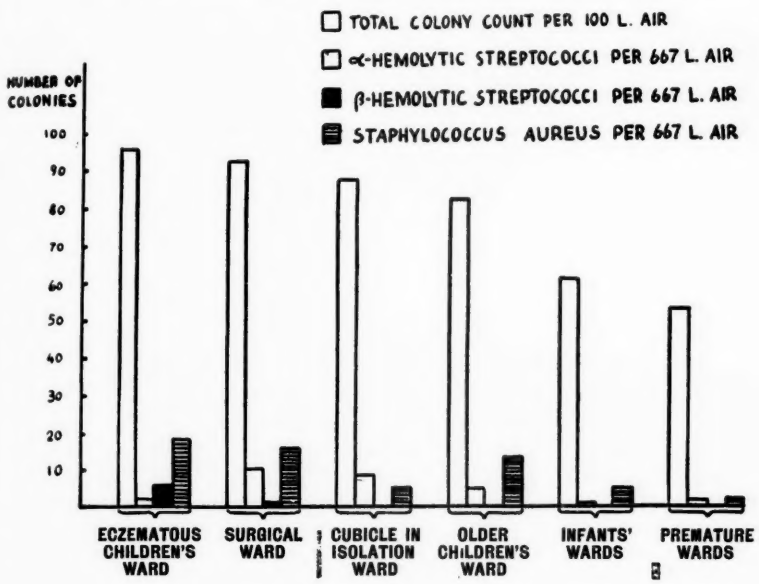


TABLE 3

MEDIAN AND MEAN OF TOTAL COLONY COUNTS OBTAINED AT DIFFERENT OBSERVATION POSTS

Observation Post	Median of Total Bacterial Count per 100 l. Air Samples	S-I Range[1]	Mean Total Colony Count per 100 l. Air Samples
Premature Wards ..	53.5	25	87
Infants' Wards	61	33	77
Older Children's Ward	82	53	109
Surgical Ward	92	25.5	98
Cubicle in Isolation Ward	87	38.5	107
Eczematous Children's Ward	95.5	46.5	142
Corridor in Eczematous Children's Unit ..	47.5	30.5	85
Nurses' Room in Eczematous Children's Unit	62.5	70	91

[1] S-I range = semi-interquartile range.

and infants'): it was lower in these wards, in which movement was generally restricted and the volume of air per patient rather large (26.5 and 23 cu.m.), than in any of the others.

The medians of the total bacterial counts obtained from the older children's unit and from the cubicle in the isolation unit were 82 and 87 (mean 109 and 107) and these are almost equally high. In the surgical ward the median was 92 and thus higher than the preceding. The greater bacterial pollution in this ward may be due to the fact that here the volume of air per child was smallest, 16 cu.m.

The highest median, 95.5 (mean 142), was obtained in the ward housing eczematous children.

The aerial pollution in the corridor of the eczematous children's unit was low compared with the wards. This was indicated by the low median — 47.5 — which is even lower than in the premature wards. The low bacterial content of the corridor may be attributed to the ventilation system: a positive pressure is maintained in the corridor by bringing in clean air. This low bacterial content can be considered evidence for the efficiency of the ventilation. In the nurses' room of the same unit the median of the total bacterial count was 62.5 and the corresponding semi-interquartile range was fairly high, 70. On this basis it can be assumed that the aerial contamination and the number of people in this room had varied greatly.

The median of all samples taken during the period of this investigation was 72 (semi-interquartile range 38) and the mean colony count *99 per 100 l. air.* In order that this result might be compared with the figures given by other observers and shown in Table 2 the mean has been calculated so as to correspond to 10 cu.ft. of air. The mean thus obtained — *281 colonies per 10 cu.ft. of air* — equals those recorded by others.

The mean colony count of the air samples from the premature wards was proportionately higher as compared with the other wards than the mean colony counts recorded by others. This is due to the fact that it was impossible for technical reasons to sample the air in the incubators; the samples were collected in wards in which there were slightly more developed premature infants in ordinary cots.

2. ALFA AND BETA HEMOLYTIC STREPTOCOCCI IN WARD AIR

For the purpose of studying the bacterial content of ward air and its importance as a vehicle of infection attempts have been made to find such specific micro-organisms as would be easy to recover from air and the absolute number of which would indicate aerial pollution. As already stated, Gordon (1903) and Wells (1935) have suggested alfa hemolytic streptococci as indicator organisms

TABLE 4

ALFA HEMOLYTIC STREPTOCOCCUS COUNTS OF WARD AIR AS RECORDED BY DIFFERENT OBSERVERS

Observer	Year	Place of Sampling	Mean Alfa Hemolytic Streptococcus Count per 10 cu. ft. Air Samples
Chapple and Kenney	1939	Pediatric Ward	3.4
Wells, Wells and Mudd	1939	Infants' and Children's Wards	0.8
		Premature Ward	0.0
		Children's Out-Clinics	13.0
Mc Donald	1940	Operating and Delivery Rooms throughout actual operating day	0.6
Long, McKhann and Cheney	1940	Premature Infants' Room	0.0
		Infants' Ward	1.83
		Children's Medical Ward	0.75

of aerial contamination. Table 4 shows the number of alfa hemolytic streptococci present in 10 cu.ft. of air as determined by different investigators. It will be seen that the older children's wards contain a greater number of alfa hemolytic streptococci than the premature wards.

On the other hand, it has been noted that the alfa hemolytic streptococcus count is directly proportional to the total bacterial count; therefore the total count might also be considered an index of the aerial pollution. Thus Buchbinder *et al.* (1945) have suggested that the total bacterial count may be used as an indirect index of nasopharyngeal contamination of schools. According to Cruicks-

hank (1946), standards of clean air should be established using either alfa hemolytic streptococci or the total bacterial content. On the other hand, Buchbinder *et al.* (1945) stress that, even if »alpha hemolytic streptococci of the air may be used as an index of human habitation their presence denoted past as well as present occupancy».

It will be remembered that Buchbinder *et al.* (1938) stated that beta hemolytic streptococci are relatively rare in normal air sampled at random. Robertson *et al.* (1944) considered the beta hemolytic streptococci in air the most suitable index of respiratory infections: numerous studies in army camps had shown, as stated in a preceding chapter, that there is a definite relationship between the number of a given type of hemolytic streptococcus present in the air and the incidence of infection with that type. The air of wards housing patients with hemolytic streptococcal infections has been studied by Hamburger *et al.* (1944): they found that the ratio of the beta hemolytic streptococcus count to the total bacterial count in such environment was at least about 1: 500, whereas Buchbinder *et al.* (1945) found this ratio to be only about 1: 4000 in normal environment.

It should still be mentioned that del Mundo and McKhann (1941) found hemolytic streptococci only occasionally in the air of children's hospitals and usually at such times organisms of the same type were recovered from the nasopharynx of patients and personnel.

Alfa hemolytic streptococci in the author's samples

Comparing the alfa hemolytic streptococcus counts of the air samples from different wards it is noticed, as shown in Table 5 and Fig. 6 (p. 45), that alfa hemolytic streptococci were most abundant in the air of the surgical ward: both the median and the mean were higher than in any of the other wards. By exposing gentian-agar plates in the sampler to 667 liters air in the surgical ward the median 10 was obtained; the semi-interquartile range was 10 and the corresponding mean 19. This result agrees with the fact that the number of alfa hemolytic streptococci in the air of a room has generally been considered an index of human habitation and pollution (Wells 1935, Buchbinder *et al.* 1945, *etc.*). As already stated, the surgical unit had the relatively lowest volume of air

TABLE 5

MEDIAN AND MEAN OF ALFA HEMOLYTIC STREPTOCOCCUS COUNTS COLLECTED AT EACH OF THE OBSERVATION POSTS

Observation Post	Median of α-Hemolytic Streptococcus Counts per 667 l. Air Samples	S-I Range [1]	Mean of α-Hemolytic Streptococcus Counts per 100 l. Air Samples	Average Volume of Air per Child
Premature Wards	2	1.5	1.7	26.5 m³
Infants' Wards ..	1	1	6.5	23 »
Older Children's W.	5	9	18	19.5 »
Surgical Ward	10	10	19	16 »
Cubicle in Isolation Ward	8	7	17	(12—110) »
Eczematous Children's Ward	2	2.5	2.8	18.5 »
Corridor in Eczematous Children's Unit	3	1.8	3.0	
Nurses' Room in Eczematous Children's Unit..	2	3.2	9.6	

[1] S-I range = semi-interquartile range.

per child and on this basis its occupancy can be regarded as the highest.

The lowest medians of the alfa hemolytic streptococcus counts were obtained from the infants' unit, premature unit, and the rooms in the eczematous children's unit. The mean corresponding to the median from the infants' wards was significantly high, 6.5, owing to the fact that these observations included one exceptionally high value (86). The median from the eczematous children's ward was significantly low considering the very high total bacterial count of this ward.

The proportionally greatest semi-interquartile range, 3.2, occurred in the alfa hemolytic streptococcus count of the samples from the nurses' room in the eczematous children's unit: this is also indicated by the considerable difference between the median 2 and the mean 9.6. The probable explanation is that the nurses were in this room only occasionally whereas the children were constantly in the wards.

The median of the alfa hemolytic streptococcus counts of all samples collected in the hospital was 3 (semi-interquartile range 2.5); the mean was 10 colonies per 20 min. run (667 l. air). When this is calculated so as to correspond to 10 cu.ft., the mean colony count is 4.1 which is of the same order of magnitude as those reported by foreign observers, shown in Table 4.

Beta hemolytic streptococci in the author's samples

Of all air samples collected from various parts of the hospital beta hemolytic streptococci were least frequent in those from the infants' wards, as evident from Table 6. In these samples the mean

TABLE 6

MEDIAN AND MEAN OF BETA HEMOLYTIC STREPTOCOCCUS COUNTS COLLECTED AT EACH OF THE OBSERVATION POSTS

Observation Post	Median of β-Hemolytic Streptococcus Counts per 667 l. Air Samples	S-I Range[1]	Mean of β-Hemolytic Streptococcus Counts per 667 l. Air Samples
Premature Wards	0	0	0.4
Infants' Wards	0	0	0.3
Older Children's Ward ..	0	0.5	3.8
Surgical Ward	1	0.7	1.6
Cubicle in Isolation Ward	0	2.2	7.1
Eczematous Children's W.	6	4	6.7
Corridor in Eczematous Children's Unit	0	0.2	1.0
Nurses' Room in Eczematous Childrens' Unit ..	0	0.7	0.8

[1] S-I range = semi-interquartile range.

beta hemolytic streptococcus count was 0.3; the median and the semi-interquartile range were 0. In these wards, as will be remembered, infants with gastrointestinal disturbances were nursed. The result seems to indicate that streptococci, at least air-borne types, play no part in the etiology of infants' gastroenteritis. This is supported by the fact that neither from the premature wards nor from »clean» infants' wards did the air samples collected during two outbreaks of nosocomial gastrointestinal infection during the

period in question yield hemolytic streptococci. On March 8, 1948, when such samplings were made in the clean infants' wards, 5 of the 14 infants nursed there showed signs of having contracted gastrointestinal infection, and on October 26, 1949, when samplings were made in the premature ward, 7 of 11 premature infants had such symptoms. Air samples were taken during these periods from all wards of these units and they displayed no significant differences compared with those taken when there was no epidemic.

In the samples from the premature wards the median of the beta hemolytic streptococcus count was the same as from the infants' wards, 0. The semi-interquartile range was also 0. But the mean colony count was slightly higher, 0.4. This may be attributed to the exceptionally high beta hemolytic streptococcus count in one of the premature wards on Oct. 27, 1948: the number of colonies was 13. Hemolytic streptococci were not cultured from any other sample from this ward during the period of the present study. This high count has been assumed to be due to the fact that an infant with septic omphalitis was being nursed in this ward at the time.

The mean beta hemolytic streptococcus count of the air samples from the surgical ward, 1.6, is relatively low considering that the volume of air per child is so small in this ward. Here older children with no manifest symptoms of respiratory infection were nursed and the beta hemolytic streptococcus count was constantly low: this is also indicated by the fact that the corresponding median 1 and the semi-interquartile range 0.7 were relatively low.

In the cubicle of the isolation unit and in the older children's unit the beta hemolytic streptococcus count was more variable and this appears from the fact that although the median values were 0, the mean counts were as high as 7.1 and 3.8 respectively. In both these units children with different respiratory infections and their sequelae were occasionally nursed and the high count is ascribed to the fact.

The median of the beta hemolytic streptococcus count obtained in the eczematous children's unit (6) and the mean (6.7) are both relatively high compared with the corresponding count at the other observation posts. From this it was assumed that beta hemolytic streptococci were constantly more frequent in this ward than in the others.

In the corridor and nurses' room of the eczematous children's unit the beta hemolytic streptococcus count was low, as appears from the relatively low mean counts 1.0 and 0.8, as well as from the median values 0 and 0.

The median of the beta hemolytic stretococcus counts of all the air samples during the period of study was 0 (semi-interquartile range 0.5), the mean count being 2.7 per 667 liters air. The ratio of beta hemolytic streptococci to the total number of colonies, as calculated on the basis of all samples collected, was 1: 244, which is noticeably higher than that determined by Buchbinder *et al.* (1945) in normal environment and referred to already.

3. STAPHYLOCOCCI OF THE AUREUS TYPE IN WARD AIR

Infections with strains of *Staphylococcus aureus* are known to be common especially among the newborn in children's hospitals. It has been stated in reports that outbreaks of pemphigus, conjunctivitis, rhinitis, *etc.*, in the newborn are due to *Staphylococcus aureus* carriers and that air may be a vehicle of infection (Bøe 1946, Muhl 1948, Allison *et al.* 1946, *etc.*). Allison and Hobbs (1947), who studied the epidemiology of pemphigus neonatorum, found that during two outbreaks of this disease in a maternity hospital an epidemic strain of *Staphylococcus pyogenes* was present in the air of the ward. Yet they could not estimate the number of these staphylococci — it appeared to be low.

It is also considered that *Staphylococcus aureus* carriers are very frequent among healthy individuals; therefore their possibilities of redispersing these bacteria into the air for instance from their skin and clothes are so great (Colebrook 1946) that this organism, when occurring in the air, cannot be regarded as an index of infections with it.

Staphylococcus aureus in the author's own samples

In several air samples from different wards the *Staphylococcus aureus* counts were seemingly paradoxical, *i.e.* considerably higher than the corresponding total bacterial counts would presuppose. No possible explanations of these exceptionally high counts can be assumed from the infections of the infants and children nursed in these wards.

In the premature, as well as in the infants' wards (see Table 7) the medians of the staphylococcus aureus counts were thus relatively low, 3 and 5, as against the corresponding mean counts, 25 and 36.

TABLE 7

MEDIAN AND MEAN OF STAPHYLOCOCCUS AUREUS COUNTS COLLECTED AT EACH OF THE OBSERVATION POSTS

Observation Post	Median of Staphylococcus Aureus Counts per 667 l. Air Samples	S-I Range[1]	Mean of Staphylococcus Aureus Counts per 667 l. Air Samples
Premature Wards	3	6	25
Infants' Wards	5	8	36
Older Children's Ward	14	12	79
Surgical Ward	16	14	27
Cubicle in Isolation Ward	5	6.5	34
Eczematous Children's W.	18	36	71
Corridor in Eczematous Children's Unit	13	13	24
Nurses' Room in Eczematous Children's Unit ..	12	14	23

[1] S-I range = semi-interquartile range.

The older children's ward and the cubicle in the isolation unit also yielded very variable *Staphylococcus aureus* counts, as indicated by the great differences between the medians, 14 and 5, and the mean counts 79 and 34.

In the eczematous children's ward *Staphylococcus aureus* was generally more frequent than in the other wards: this is evident from the fact that the median 18 (and the mean 71) are high compared with the counts from the other wards.

As appears from the median values in Table 7, yellow staphylococci were on the whole less frequent in the air of infants' wards than in those of older children, with the exception of the eczematous children's wards.

What has been stated above shows that the number of air-borne yellow staphylococci was variable. This may be attributed to the fact that, as referred to already, carriers spreading these organisms are so frequent among healthy individuals that, at least

without more detailed analysis, the air-borne staphylococci isolated by the present method cannot be considered definitely related to the infections due to these organisms.

The median of the *Staphylococcus aureus* count of all air samples (667 l. air) collected during the year under review was 8.5, the semi-interquartile range 12, and the corresponding mean 38.

4. INFLUENCE OF SEASONAL VARIATIONS AND OF THE RELATIVE HUMIDITY OF AIR ON THE BACTERIAL CONTENT OF WARD AIR

The bactericidal effect of light as well as seasonal influences in the origin of epidemics of various types have long been considered established facts. Several investigators have shown that there is a definite correlation between seasonal changes in lighting and ventilation conditions on the one hand, and air-borne bacteria and hospital infections on the other. Thus Buchbinder *et al.* (1941) and Buchbinder (1942) demonstrated that natural daylight and sunlight, especially in spring and summer, have a lethal effect on air-borne bacteria in rooms. Garrod (1944) says that good natural daylight is a very potent factor in the control of air-borne infections because it destroys bacteria even through closed windows. Torrey and Lake (1941), as well as Walter and Hucker (1942), state that the lowest incidence of streptococci in inhabited rooms of different type is during the summer months.

M. Wells (1944) considers that the seasonal periodicity of respiratory infections is due to domestic ventilation which, in its turn, varies with the outdoor temperature. Peters (1946) says that the wartime black-out in hospitals made ventilation of wards more difficult and so increased the possibilities of cross-infection. And, because the bacterial pollution of operating rooms is smaller in summer, Hart (1942) suggests that at least large clean operations should as far as possible be done during the warmer months.

The relative moisture and the temperature of wards are known to affect the well-being of patients. Basing himself on Mollier's well-known diagrams of comfort-zones Fischer (1949) states that people are more comfortable in room environments at a temperature of 18 to 22° C., the relative humidity of air being 70 to 40 per cent. According to Chapple (1942), who studied especially the influence of external factors on the well-being of premature infants,

it seems to be established that premature infants do better in high humidities. In a report on cross-infection in children's hospitals Allison *et al.* (1946) express the view that the temperature of wards should not be above 15.6° C. except in nurseries for the newborn.

The relative humidity of air has been found to be of decisive importance for the germicidal action of aerosols (Baker and Twort 1941). Glycol vapour is considered most effective at relative humidities of 45 to 70 per cent (Puck *et al.* 1943), of 40 to 50 (Hamburger *et al.* 1945), and of 40 to 60 per cent (Robertson 1943).

Wohlfeil (1937) studied the effect of air-moisteners on the bacterial content of air in rooms. He arrived at the conclusion that artificial moistening of air affects air-borne bacteria in rooms so slightly that it is hygienically negligible. On the other hand, Wells and Riley (1937) noted that the numbers of *E. coli* in the air of textile mills decreased in higher humidities and Loosli *et al.* (1943) demonstrated that influenza virus A was killed rapidly in humid air. Dunklin and Puck (1948) consider that there is a narrow range of relative humidity in the vicinity of 50 per cent, which is as such lethal for micro-organisms; this range varies slightly with the type of bacteria.

It is thus clear that the relative humidity of air and seasonal variations are closely related to aerial pollution and the spread of infections; accordingly the median and the mean of the total bacterial counts for the different seasons were determined and the average of the relative humidities calculated. These are shown in Table 8.

TABLE 8

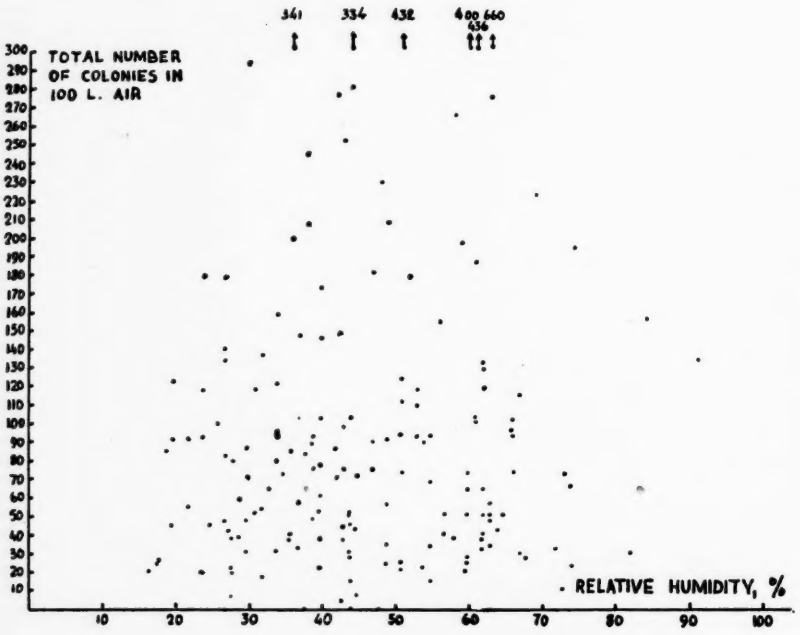
MEDIAN AND MEAN OF THE TOTAL BACTERIAL COUNTS IN THE DIFFERENT SEASONS, AND THE AVERAGE RELATIVE HUMIDITIES

Season	Median of Total Bacterial Count (100 l. Air Samples)	S-I range[1]	Mean of Total Bacterial Count (100 l. Air Samples)	Average Relative Humidity %
Spring (March, April, May)	51.5	23	79.5	39.2
Summer (June, July, Aug.)	45	26.5	57	54.1
Autumn (Sept., Oct., Nov.)	84	38.5	112	51.7
Winter (Dec., Jan., Febr.)	83	37	99	34.1

[1] S-I range = semi-interquartile range.

Fig. 7.

TOTAL COLONY COUNTS OF THE AIR SAMPLES IN RELATION TO RELATIVE HUMIDITIES



As evident also from this table the bacterial content of air was lowest during the summer months when, on the other hand, the relative humidity was highest because rooms are heated least and natural ventilation is at a maximum. The bacterial content was highest in autumn and winter, which agrees well with the finding that respiratory infections are most prevalent in hospitals during the first few and last few months of the year (Helve 1948).

The question seemed of interest whether there was any direct relationship between the aerial contamination and the relative humidity of air in the different parts of the hospital during the year under review; particularly whether Dunklin and Puck's (1948) assertion already referred to above (that there is a range of relative humidity in the vicinity of 50 per cent which is lethal for bacteria) plays any important part in these samples. Fig. 7 shows the changes taking place in the total bacterial content in different relative humidities. On the basis of the results no correlation could be

demonstrated between the humidity of air in wards and their total bacterial content. Also, no evident decrease appeared in the total bacterial content in humidities of about 50 per cent.

5. INFLUENCE OF THE ROUTINE PROCEDURES AT DIFFERENT TIMES OF DAY ON THE BACTERIAL CONTENT OF AIR

It has been shown in numerous scientific reports that the bacterial content of air in wards increases as a result of various routine hospital activities. In 1936 White showed that for instance bedmaking and sweeping of floors cause an increase in air-borne hemolytic streptococci. Allison and Brown (1937) found the same thing in scarlet fever wards and assumed that it was due to air currents produced by the staff's movements.

Lojander (1934) studied the bacterial content of air in a gymnasium and found that movement increases aerial contamination. He also showed that such an increase, brought about by movement, was usually considerably reduced in about 40 minutes. He concluded, then, that air-borne bacteria occur in large dust particles which settle rapidly and this would account for the rapid return of the bacterial content to the earlier level.

Cruickshank and Godber (1939) have also noted that dry sweeping raises the dust and therefore increases the bacterial content of air. Similarly van den Ende *et al.* (1940) have stated that »bedclothes and floor dust in the vicinity of patients suffering from certain diseases of the respiratory tract constitute reservoirs from which infectious agents are redispersed into the air on agitation». Thomas and van den Ende (1941) noted that even straightening the bedclothes of one bed suffices to raise the bacterial count of the surrounding air by one hundred times. Robertson *et al.* (1944) and Hamburger *et al.* (1944) have also pointed out that the beta hemolytic streptococcus count of ward air is low when there is no activity in the ward but increases as a result of different procedures, such as bedmaking. According to Buchbinder *et al.* (1945) sweeping increased the number of air-borne bacteria in a ward threefold, but the counts returned to the pre-sweeping level within one hour. In connection with their studies of the sanitary conditions in dressing rooms Bourdillon and Colebrook (1946) stated that the shaking of pyjamas or bedclothes, which have been

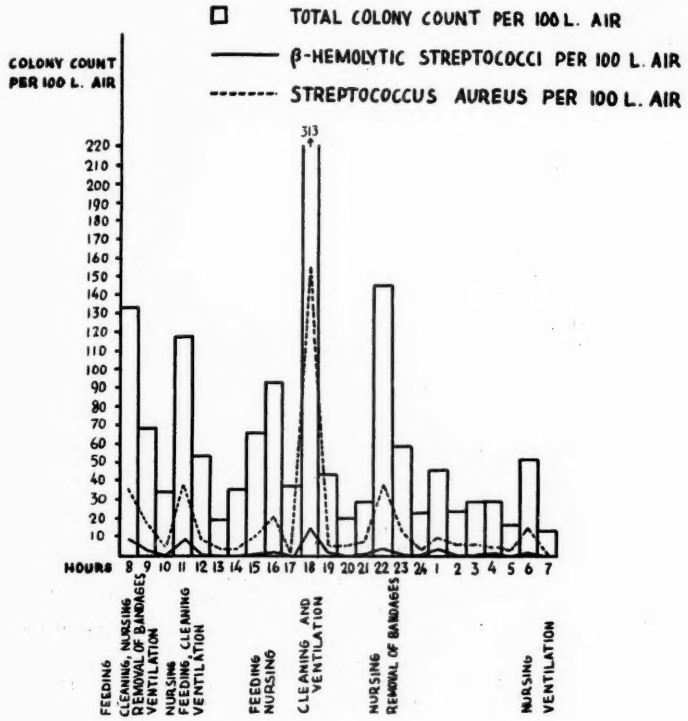
used for some hours, causes large clouds of bacteria, often pathogens, for instance *Staphylococcus aureus*, to be released. Therefore they stress that unoiled bedclothes should not be allowed in any surgical dressing rooms. Pulvertaft (1947) reports his personal observation that a hospital ward normally has about 50 bacterial particles per cubic foot of air but during bedmaking the number rises to 2000—3000. Löfström (1947) has presented a diagram of the variations in the bacterial content of air in a children's hospital ward. This diagram shows that the number of bacteria raised by different routine activities clearly decreases as a result of natural ventilation.

Thus there is ample evidence that the number of certain bacteria originating in the nasopharynx or skin of patients, as well as the total bacterial count, increase owing to different activities in the wards. Yet the question seemed interesting to what extent the changes in the number of air-borne hemolytic streptococci and *Staphylococcus aureus* resemble those in the total bacterial content. For this purpose the bacterial content of air was followed hourly for 24 hours in an eczematous children's ward in which the bacterial counts had proved to be high, and also in a corridor of the Outpatient Department where the numbers of air-borne bacteria are also, for obvious reasons, high. In addition, the variations in the total bacterial content were studied in a double ward housing infants with gastrointestinal infections; here the bacterial count had generally been low. This was done in order to reveal whether, in a ward of this type, the variations in the total bacterial content are similar to those in the types of ward mentioned above.

In one of the wards for eczematous children 100 liter air samples were taken at hourly intervals for 24 hours on May 23 to 24, 1949, on blood agar plates; from these the total bacterial count and the beta hemolytic streptococcus and *Staphylococcus aureus* counts were determined. Fig. 8 shows the variations in these counts at different times of day. It appears that different routine procedures, such as cleaning and also the removal of bandages — the effect of which is most clearly noticeable in the samples collected at 10 p.m. (22 hours) — distinctly increased the aerial pollution; on the other hand, natural ventilation reduced it. The numbers of beta hemolytic streptococci and pathogenic staphylococci in the air varied with the total bacterial content.

Fig. 8.

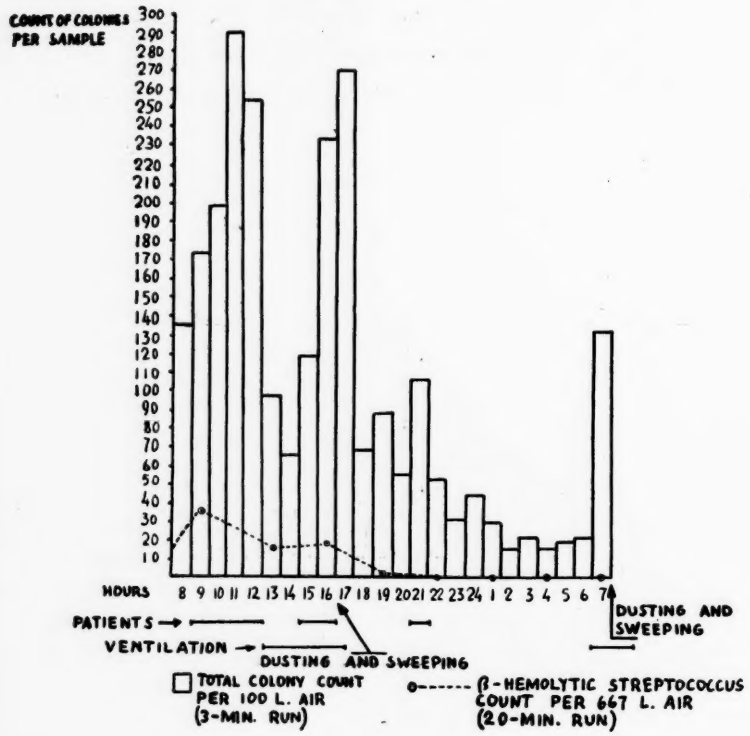
TOTAL COLONY COUNT, HEMOLYTIC STREPTOCOCCUS AND STAPHYLOCOCCUS AUREUS COUNTS IN THE AIR OF A WARD HOUSING ECZEMATOUS PATIENT (MAY 23—24, 1949)



In the corridor of the Outpatient Department the changes in the total bacterial content and in the number of beta hemolytic streptococci were followed on December 3 to 4, 1948, and those in the *Staphylococcus aureus* and total bacterial counts on December 10 to 11, 1948. For the total bacterial counts 100 liter air samples were taken every hour for 24 hours on blood agar plates; for the beta hemolytic streptococcus and *Staphylococcus aureus* counts 20-min. samples (667 liters air) were collected, the former on gentian agar, the latter on Chapman agar every 3 to 4 hours. In the diagrams the total bacterial counts are indicated by columns and the beta hemolytic streptococcus counts (in 667 liters air) by a broken line (Fig. 9). The *Staphylococcus aureus* counts are

Fig. 9.

VARIATIONS IN TOTAL COLONY COUNT AND IN NUMBER OF β-HEMOLYTIC STREPTOCOCCI DURING 24 HOURS (DEC. 3—4, 1948) IN CORRIDOR OF OUT-PATIENT DEPARTMENT

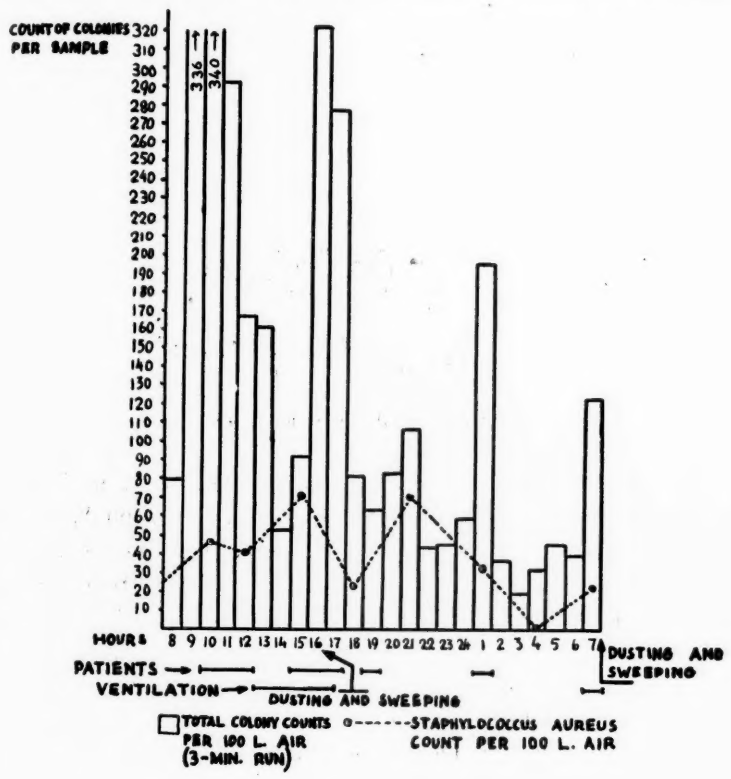


also represented by a broken line but they are reduced so as to correspond to 100 liters air (Fig. 10).

Figs. 9 and 10 show that, just as in the wards, the bacterial counts were high in the corridor of the Outpatient Department during sweeping and dusting, and in addition during consultation hours, and that also here natural ventilation caused a distinct reduction in the number of air-borne bacteria. The variations in the beta hemolytic streptococcus counts, as well as those in the *Staphylococcus aureus* counts, seem also here to be on the whole similar to those in the total bacterial content; staphylococci were much more numerous than streptococci but there were no extremely high staphylococcus counts.

Fig. 10.

VARIATIONS IN TOTAL COLONY COUNT AND IN STAPHYLOCOCCUS AUREUS COUNT DURING 24 HOURS (DEC. 10—11, 1948) IN CORRIDOR OF OUTPATIENT DEPARTMENT

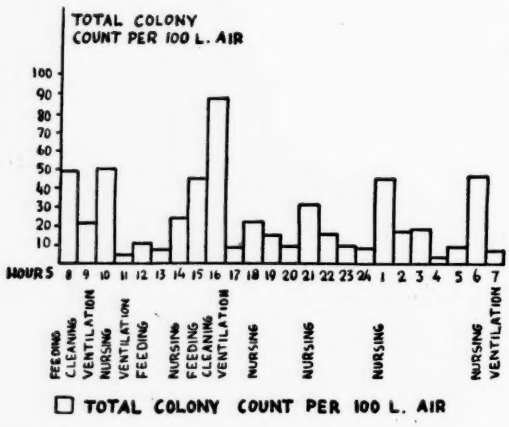


In a ward, in which two infants with gastrointestinal infections were nursed, total bacterial counts were determined every hour during 24 hours on Sept. 23 to 24, 1949, by taking 100-liter air samples on blood agar plates. It will be seen in Fig. 11 that the total bacterial content, which was always remarkably low compared with the ward described above, increased also here as a result of various routine activities and decreased during natural ventilation. In this ward attempts were made to study the variations in the *E. coli* content of the air by taking samples on Drigalski-Conradi plates, but the counts were so low that the attempts were futile.

The results showed that activities which raised the dust in the ward, for instance bedmaking and sweeping — in spite of the

Fig. 11.

HOURLY VARIATIONS IN TOTAL BACTERIAL CONTENT IN WARD HOUSING 2 INFANTS WITH GASTROINTESTINAL INFECTIONS, SEPT. 23—24, 1949



fact that a wet cloth was used — caused the most noticeable increase in the bacterial pollution of the air. The high bacterial content generally fell again within one hour. This indicates that the bacteria in a ward originate chiefly in its dust. In the Outpatient Department where the number of patients is high compared with the wards, the highest counts were obtained during consultation hours, but subsequent cleaning activities again increased the bacterial content almost as much.

It was apparent that natural ventilation clearly reduced the bacterial content of ward air. To judge from the diagrams the fact that the central ventilation plant is not in operation during the quiet hours from 8 p.m. till 7 a.m. did not affect the bacterial pollution of ward air.

6. POSSIBLE INFLUENCE OF THE HOSPITAL STAFF ON AIR CONTAMINATION IN WARDS

There is definite evidence that healthy streptococcus and staphylococcus carriers are able to spread infection. Colebrook (1933) reports that about 5 to 30 per cent of normal people may be throat carriers of hemolytic streptococci but thinks that infection will not be very readily conveyed by the healthy throat carrier. As already mentioned, he was the first to recognize that there are

dangerous as well as harmless carriers and he believed nasal carriers to be a greater danger than throat carriers. Hamburger *et al.* (1944, 1945, 1949) also noted that nasal carriers with a positive nose culture are most dangerous. According to Colebrook (1946) 1 to 2 per cent of streptococcus carriers are nose carriers and dangerous throat carriers. Cook and Munro-Ashman (1949) also stress the importance of the early detection and isolation of such dangerous nasal carriers from the point of view of prevention of infection.

TABLE 9

HEMOLYTIC STREPTOCOCCI AND GROUP A HEMOLYTIC STREPTOCOCCI IN CARRIERS ACCORDING TO DIFFERENT INVESTIGATORS

Observer	Year	Subjects Studied	Number of Subjects	Hemolytic Streptococci %	Group A Hemolytic Streptococci %
Colebrook	1933	Healthy adults	—	5—30	—
Hare	1935	Adults	—	—	7
Frish	1938	Undergraduates	621	16.2	6.4
Ernst	1942	Hosp.personnel	427	—	2.1
Kalbak	1942	Healthy adults	100	7	5
Schwentker et al.	1943	15-year-olds	7802	23	14
Gaustad	1945	Healthy school children	125	40.8	—
Pike and Fashena	1946	Children	900	42	25
Lagercrantz	1948	Adults	309	14.2	10
Packalén	1949	Patients with tuberculosis	386	52.8	—

The number of hemolytic streptococcus carriers varies considerably among the human population owing to different factors. Van Ravensway (1944), for instance, says that geographical factors, seasons and weather conditions affect the carrier rate. It has been demonstrated that this rate is higher during the winter than during the summer months (Long *et al.* 1939, Peacock *et al.* 1939, *etc.*). Also, the incidence of carriers is said to be higher during epidemics of streptococcal infections (van Ravensway 1944, Hamburger 1949, *et al. etc.*). Table 9 illustrates the incidence of healthy throat carriers of hemolytic streptococci as recorded by different observers, and in addition, at the bottom of the table, of throat carriers among

tuberculosis patients. This table gives the total beta hemolytic streptococcus count and/or the Group A streptococcus count (Lancefield). According to the opinion of Hare (1940), later confirmed by several investigators, 95 per cent of all streptococci causing beta hemolysis on blood agar are of Lancefield's serological type A. As will be seen in this table, there are considerable differences in the numbers of healthy streptococcus carriers given by different workers.

As regards the occurrence of *Staphylococcus aureus* in healthy individuals it should be mentioned that according to McFarlan (1938) the main reservoir of these staphylococci is generally considered to be the nose. Gillespie *et al.* (1939) examined 159 medical students and found that 43.4 per cent were nasal carriers of virulent strains of staphylococci, 19.5 per cent were skin carriers and 12.6 were double carriers. Williams (1946) considers that among the healthy population there may be about 50 per cent permanent nose carriers and as many as 80 to 90 per cent temporary carriers. In studying the possible sources of infection in pemphigus neonatorum Parker and Kennedy (1949) obtained *Staphylococcus aureus* from the noses of one-half of the staff in the first two swabbings and expressed their view that the ultimate source of the organisms responsible for the infection were the nasal passages of adults. On the other hand, they think that the main path of infection was from infant to infant.

Regarding the part played by the personnel in the spread of cross-infection in children's hospitals Ylppö (1935) has stated that isolation of the patients from each other does not help, if spread of infection from nurses and doctors cannot be effectively prevented at the same time; the newborn and premature wards should as far as possible be staffed by older nurses and such nurses as have proved especially resistant to respiratory infections. Long *et al.* (1940) studied cross-infections in children's hospitals and concluded that the source of most infections were the hospital personnel and visitors rather than fellow patients. Colebrook (1946) has called attention to the danger from possible carriers on the hospital staff, stating that throat swabs should be taken before nurses are transferred for duty in clean wards.

Rosenstern (1942) carried out studies in an Adoption Nursery and found that in the majority of cases the infant and not the nurse was the source. According to Loosli and Smith (1949), who also

investigated the question of cross-infection in children's hospitals, such cases were in each instance due to another patient in the ward and there was no reason to suspect the source to be among the hospital personnel or visitors.

In order to discover the extent to which throat and especially nose carriers are present in a children's hospital in Finland, throat and nose swabs of 336 members of the staff were taken by the usual method on blood agar between November 5, 1948, and April 20, 1949. The streptococci yielded by these swabs could not be typed: they were only differentiated on the basis of beta hemolysis. Similarly the staphylococci of aureus type were classified on the basis of pigment formation in the colonies (Packalén and Bergqvist 1947).

Table 10 shows the number of throat and nose carriers as percentages of the total number of swabs taken.

TABLE 10

HEMOLYTIC STREPTOCOCCI AND STAPHYLOCOCCUS AUREUS IN THROAT AND NOSE SWABS OF HOSPITAL PERSONNEL IN THE CHILDREN'S CLINIC

	Number of Samples	Culture Positive for the Hemolytic Streptococcus	Culture Positive for Staphylococcus Aureus
Nose swabs	336	0.3 %	11.9 %
Throat swabs	336	33.6 %	1.9 %

The throat swabs yielding hemolytic streptococci were classified according to the number of colonies on the plates using Hamburger's (1944) classification (See Table 11).

TABLE 11

THROAT CULTURES POSITIVE FOR THE HEMOLYTIC STREPTOCOCCUS CLASSIFIED ACCORDING TO NUMBER OF COLONIES ON PLATE

	1 to 10 Discrete Colonies	10 to 40 Discrete Colonies and a Definite Zone of Beta Hemolysis	Heavy Growth or Pure Culture of Hemolytic Streptococci
Number of samples in the different groups	42	60	11
% of positive cultures ..	37.2 %	53.1 %	9.7 %
% of all throat cultures examined	12.5 %	17.8 %	3.3 %

As shown in Table 11, only 3.3 per cent of the throat cultures revealed heavy growth of beta hemolytic streptococci and 12.5 per cent showed less than 10 hemolytic colonies.

The 336 subjects studied thus included a considerably greater number of streptococcus carriers than of *Staphylococcus aureus* carriers. The high incidence of streptococcus carriers is partly due to the fact that most of the swabs were taken during the winter months, when, as pointed out previously, the streptococcus carrier rate is high. Over one-third, or 33.6 per cent, of those examined were throat carriers. On the other hand, their number included only one nasal carrier. Staphylococci of the aureus type were very rarely found, compared with the results of earlier observers. Nose carriers of *Staphylococcus aureus* amounted to 11.9 per cent of those examined, and throat carriers to 1.9 per cent.

Hamburger *et al.* (1949) found that in military training organisations in the U.S. the ratio of nose to throat carriers was 1: 43.8 when the incidence of streptococcal infection was low, and 1: 1.3 when that incidence was high. In the present material the ratio of nose to throat carriers was 1: 112. This ratio is even smaller than that obtained by Hamburger *et al.* when the attack rate was low.

7. HOSPITAL PLANNING AS REGARDS THE TRANSMISSION OF AIRBORNE INFECTIONS

Watkins and Lewis-Faning (1949) studied the incidence of cross-infection in 14 children's hospitals in Britain, attempting to correlate factors possibly responsible for variation in this incidence. These factors included ward architecture, equipment, nursing, *etc.* As a result of their studies they were not able to give any advice to hospital planners on the value of common architectural practices in preventing cross-infections.

On the other hand, it has been known for a long time that infections spread in hospitals in the direction of the air current (Meyer 1913, Dzialoszynski 1926). Wheeler and Duckett Jones (1942) reported that a highly fatal hemolytic streptococcal infection in a rheumatic fever hospital spread by way of the stairways from the lowest floor to the upper floors carried by

TABLE 12

TABLE SHOWING STAIRWAYS AND WARDS IN THE CHILDREN'S CLINIC, THE MEDIAN (M) (OF THE TOTAL COLONY COUNTS) SEMI-INTERQUARTILE-RANGE (S-I) AND MEAN (K) OF AIR-SAMPLES

	Unit	Stairway I	Unit	Stairway II		
4th floor	Newborn Wards	M 62.5 S-I 21 K 67	Surgical Wards	M 129 S-I 60.5 K 159		
						Stairway III
3rd floor	Wards for Infants With Diarrhoea	M 79.5 S-I 22 K 78	Premature Wards	M 122 S-I 38.5 K 144	Lecture Hall	M 69.5 I-S 39 K 77
2nd floor	Infants' Wards	M 57.5 S-I 31 K 74	Roentgen etc.	M 119 S-I 57 K 156	Outpatient Department	M 164.5 I-S 96.5 K 236
1st floor	Older Children's Wards	M 53 S-I 31 K 76	Kitchen Staff Restaurang	M 23.5 S-I 16 K 41		

The figures show the median and mean of the total colony counts in ten 100 l. air samples. The samples were always taken in the same order and successively

upward air currents. It was later proved that this was the avenue of infection: streptococci of the same type as the infecting organism were found in the stairways.

As infecting organisms and cross-infections may thus spread by way of the corridors and stairways connecting the different wards, it seemed that the placing of the wards might be of great importance. To throw light on this question air samples were also collected from the stairways of the Children's Clinic, *i.e.* 10 successive samples from each landing where glass doors lead to the wards (Table 12). The figures in the table show the median and the mean of the total colony counts on each landing.

Table 12 shows that there were no marked variations in Staircase No. 1, adjoined chiefly by wards on both sides. In Stairway No. 2 is a door leading to the Outpatient Department and the bacterial content is therefore high. On the first floor is the main entrance to the hospital and from here a corridor leads to the kitchen and the staff restaurant. In spite of the fact that there is usually much movement here, the counts yielded by all the successive air samples were lower than those obtained in the same staircase on the upper floors. On the third floor — above the Outpatient Department — is the entrance to the Premature Unit; there is usually less movement here and the bacterial content was on an average slightly higher than on the floor below. On the top floor, where the Surgical Unit is, the bacterial content was even higher than outside the Outpatient Department on the second floor, in spite of the fact that there is much less movement.

Stairway No. 3 is in a part of the building which is only two-storied. Here is the main entrance to the Outpatient Department and above is the Lecture Hall; the bacterial counts were always considerably higher downstairs. On the upper landing there is generally little movement except at lecture time.

In Staircase No. 2 the air current may conceivably rise upwards carrying with it bacteria from the Outpatient Department to the upper floors because of the warmer air escaping from the kitchen. This is supported by the fact that in Staircase 3, which also has an entrance to the Outpatient Department, the bacterial content was so much lower upstairs.

For comparison similar samplings were made also in the stairways of the Children's Castle (See description on p. 72). The air

contamination was on an average similar on all floors (Table 13). This institution has no Outpatient Department and the kitchen is farther away from the wards and thus the bacterial content of the central stairway is not affected in the same way.

TABLE 13

TABLE SHOWING STAIRWAY AND WARDS OF CHILDREN'S CASTLE, THE MEDIAN OF THE TOTAL COLONY COUNT (M), SEMI-INTERQUARTILE-RANGE (S-I) AND MEAN (K) OF AIR SAMPLES

	Wards	Stairway	Wards
4th floor	Laboratory	M 90.5 S-I 7 K 91	Laboratory
3rd floor	Premature Wards	M 76.5 S-I 11 K 90	Infants' Wards
2nd floor	Play-age Wards	M 85.5 S-I 27.5 K 89	Play-age Wards
I st floor	Isolation Wards	M 76 S-I 13 K 91	Isolation Wards
Ground floor	Entrance Hall	M 86.5 S-I 25.5 K 102	

The figures show the median and mean of the total colony counts in ten 100 l. air samples. The samples were always taken in the same order and successively.

K. AIR SAMPLES COLLECTED ELSEWHERE THAN IN THE CHILDREN'S CLINIC

1. SAMPLES FROM THE PATIENTS' HOMES

Horewood (1931) recovered streptococci from dust samples collected in various households and Finland (1942), who studied the spread of streptococcal infections in families, says that the prevention of secondary infections is more difficult in private households because the infecting agent has usually spread throughout the household by the time it is first discovered.

In order to find out whether the environment of children and infants is cleaner in their homes or in hospital, from the point of view of bacterial contamination of air, samples were taken from 13 homes in which there are children. The households thus selected were in different parts of the town and differed in size and number of occupants. The sampling was made in the room in which the children usually are and in each household one hour after the time appointed, so that there should be no preparatory dusting just before the sampling. The samples from all these households were collected during February 1949. As will be seen from Table 14 and 15, the total bacterial count was fairly high in all the samples, especially those from homes in which the volume of air per person was low. The median of the total bacterial count was about 592 and the mean about 786, and these are much higher than the median and mean values obtained in the Children's Clinic during the winter months, *i.e.* 83 and 99 respectively. The number of hemolytic streptococci was roughly comparable to the total bacterial counts: their number was highest in the samples yielding high total bacterial counts. Exceptions are the samples taken in the twelfth household: here the hemolytic streptococcus count was relatively high

TABLE 14

AIR SAMPLES FROM PATIENTS' HOMES

Number of Occupants	Volume of Air per Person Cu.m.	Relative Humidity %	Temperature C°	Total Colony Count (100 l. Air)	α-Hemolytic Streptococci (667 l. Air)	β-Hemolytic Streptococci (667 l. Air)	Staphylococcus Aureus (667 l. Air)
6 persons (2 children)	6.6	41	18	c. 2000	c. 130	c. 25	c. 900
7 » 5 »	11	34	18	c. 1100	48	17	48
8 » 6 »	13	51	14	c. 1200	32	2	c. 250
5 » 3 »	22	41	18	c. 2000	c. 300	c. 30	92
4 » 2 »	32	43	19	137	14	0	0
7 » 4 »	33	51	20	592	c. 320	4	c. 460
5 » 3 »	39	42	23	358	53	3	41
4 » 2 »	42	44	20	209	14	2	0
5 » 3 »	47	66	20	392	13	0	9
6 » 4 »	50	51	20	c. 1000	59	4	154
6 » 4 »	50	46	22	635	12	0	32
7 » 5 »	61	67	21	376	19	13	221
4 » 2 »	75	46	21	191	46	0	167
			Median	c. 592	c. 49	c. 3	c. 92
			Mean	c. 786	c. 86	c. 8	c. 183

TABLE 15

COMPARISON OF AIR SAMPLES FROM PATIENTS' HOMES AND CHILDREN'S CLINIC COLLECTED AT THE SAME TIME OF THE YEAR, SHOWING MEDIAN (M), SEMI-INTERQUARTILE RANGE (S-I) AND MEAN (K) OF TOTAL COLONY COUNTS, α- AND β-HEMOLYTIC STREPTOCOCCUS COUNTS, AND STAPHYLOCOCCUS AUREUS COUNTS

	Total Colony Counts per 100 l. Air Samples			α-Hemolytic Streptococci in 667 l. Air Samples			β-Hemolytic Streptococci in 667 l. Air Samples			Staphylococcus Aureus in 667 l. Air Samples		
	M	S-I	K	M	S-I	K	M	S-I	K	M	S-I	K
Samples from private households	c. 592	c. 433	c. 784	c. 49	c. 47.5	c. 86	c. 3	c. 15	c. 8	c. 92	c. 107	c. 183
Samples from Children's Clinic	83	37	99	7	3	2	0	0.5	0.7	17	9.5	25

(13) compared with the total count. This high streptococcus count may be due to the fact that there was an eczematous child in the family. The number of alfa hemolytic streptococci was considerable in all these samples and this is understandable seeing that their number can be regarded as an index of human habitation (Wells 1935, Buchbinder *et al.* 1938 and 1945). The median of the beta hemolytic streptococcus count per sample (667 liters air) was c. 3 and the mean c. 8, the median of the alfa hemolytic streptococcus count was c. 49 and the mean c. 86 in the same volume of air. In the Children's Clinic these figures were respectively 0 and 0.7, and 7 and 2.

The *Staphylococcus aureus* counts varied greatly also in the samples from private households and this may again be attributed to their frequency in normal human environment. The median of the staphylococcus count was c. 92 and the mean c. 183, as against 17 and 25 in the Children's Clinic.

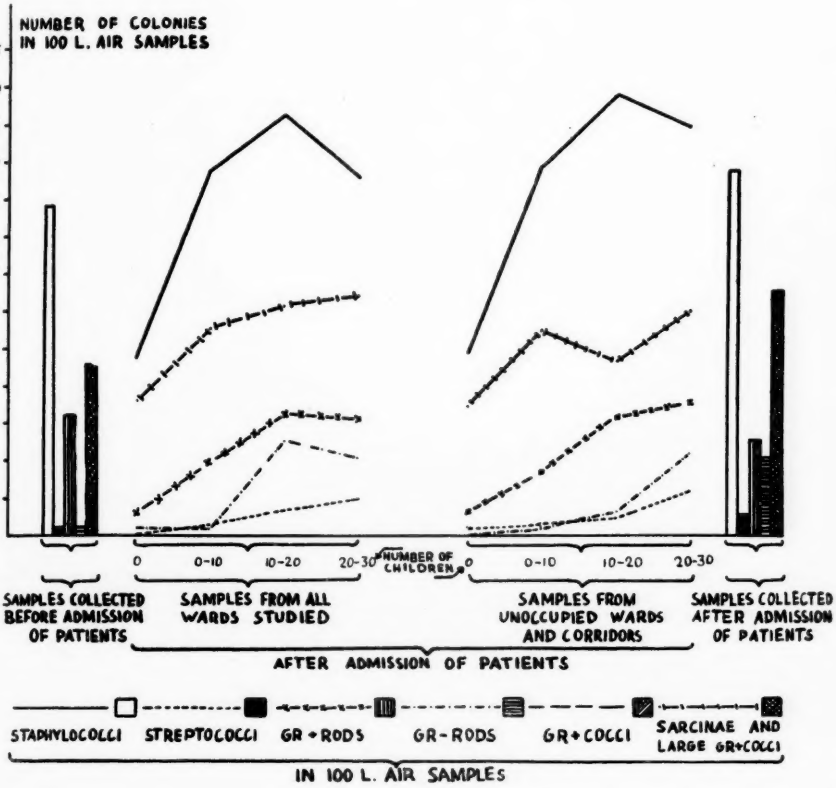
2. SAMPLES FROM THE NEW CHILDREN'S CASTLE ON COMPLETION OF THE BUILDING AND AFTER ADMISSION OF PATIENTS

Sauer, Minsk and Rosenstern, in 1942, published the results of their studies dealing with the efficiency of different measures to control the spread of infection in a new Nursery for young infants. These different measures could be applied at the time the building was completed and after the work had started, and they were then compared. The design of the Nursery is such that infants can be nursed in wards equipped with ultraviolet light, in barrier-units — in which a glass wall separates the infant from the nurse — and in wards where air-conditioning provides an exchange of air every six minutes. It appeared that air-conditioning there did not prevent cross-infections of the respiratory tract, and that mechanical barriers and germicidal light were more efficient.

The new Children's Castle is an institution chiefly for the training of nurses. The number of infants and children nursed here is 150 and they are usually healthier than in the Children's Clinic. The institution has six departments which are situated as shown in Fig. 9 (p. 69). The staff numbers 184, including 150 trainees. The same hygienic principles are followed with regard to sweeping, dusting, ventilation, nursing staff, and visitors as in the Children's

Fig. 12.

AIR SAMPLES FROM CHILDREN'S CASTLE BEFORE AND AFTER ADMISSION OF PATIENTS



Clinic. The doors between the corridors and the wards are also here usually kept closed and there are glass windows between the wards and the corridors, and in addition apertures for passing nursing utensils, food, *etc.*, into the wards without having to open the doors. These apertures are also usually kept closed.

The Children's Castle was opened to patients on November 13, 1948. Fig. 12 shows the changes which took place in the bacterial counts after admission of patients: samples were taken from all rooms of four units when the patients in each of the units numbered 0—10, 10—20, and 20—30, and in addition from unoccupied wards and corridors of the same units. The first columns on the left indicate the average of 50 air samples taken before the admis-

sion of patients in various parts of the institution (median of the total bacterial count 72, semi-interquartile range 25.5, and mean 82). The columns show that chiefly staphylococci and sarcinae were recovered. On the right are columns showing the average of 150 air samples collected after admission of patients from various parts of the institution (median of total bacterial count 102, semi-interquartile range 43, and mean 120).

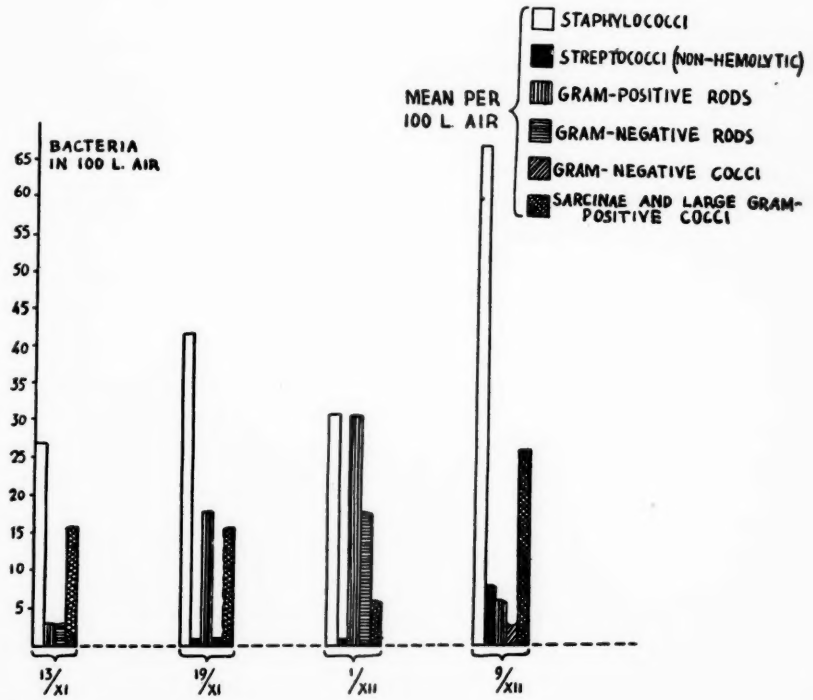
A comparison of the bacterial flora before and after admission of patients reveals that it changed fairly little and that the greatest increase was in the proportion of gram-negative rods and streptococci. Also after admission of patients the majority of air-borne bacteria were staphylococci and sarcinae. In relation to other types of bacteria the number of gram-negative cocci was so low that it was not possible to show it in the diagram.

The diagrams in the centre illustrate the variations in different types of bacteria as the number of patients admitted to the wards increased from 0 to more than 20. In the curves on the left is seen the increase in all the wards studied and on the right in the unoccupied wards and corridors. In both diagrams the counts corresponding to 0 patients were obtained by sampling the air of two units which could be kept strictly locked for two days before the admission of patients. It appears from these diagrams that the changes in the bacterial flora were rather similar in the whole units, also in the empty wards and corridors. The number of air-borne streptococci in the samples from the units studied increased in proportion to the number of patients admitted, similarly the gram-negative rods. Other bacteria did not increase essentially in number when the number of patients in the wards rose above 20.

When these samples were being collected the air-conditioning plant was not yet in operation and in these wards therefore only natural ventilation was used. A comparison of the samples from the Children's Castle and from the Children's Clinic shows that in the latter, where the central ventilation plant was in operation, the bacterial content of the wards and the corridors differed considerably, whereas in the Children's Castle the bacterial flora was almost similar in the different parts studied. It will be remembered that in both institutions the doors between the wards and the corridors were usually kept closed. From this it is evident that a

Fig. 13.

AIR SAMPLES FROM UNOCCUPIED ISOLATION WARDS IN CHILDREN'S CASTLE



ventilation system maintaining a positive pressure in the corridors effectively prevents bacteria entering the corridors from the wards. On the other hand, it must be kept in mind that, in the Children's Castle with its proportionately larger personnel, there is more movement in the corridors which also, naturally, increases the bacterial contamination.

In the unit for children of play age, on the second floor, the first three wards had been kept locked after the completion of the building, except for natural ventilation and sweeping and dusting just as in the other wards. In Fig. 15 is seen the mean of the bacterial counts of air samples collected on different days from the three unoccupied wards. The diagram indicates that even in such locked rooms great variations appear in the bacterial content owing to air currents, sweeping and dusting, varying humidity, *etc.* The bacterial content of these rooms was lowest on November 13, 1948,

when no patients had yet been admitted to the other wards of this unit (this suggests that even locked doors do not entirely prevent the mixing of atmospheres). In addition, it is seen that the total number of streptococci — all non-hemolytic strains — was directly proportional to the total bacterial count also in these samples taken from the unoccupied wards.

L. OTHER POSSIBLE SOURCES OF INFECTION IN THE ENVIRONMENT

Thanks to aseptic nursery technique, possibilities of contact infection are now practically eliminated, as Dick *et al.* (1928) have pointed out. On the other hand, secondary infections in hospitals are still reported in which there is conclusive evidence of contact spread. Kenny and Barber (1944) described an outbreak of hemolytic streptococcal infection, and by typing streptococci they were able to demonstrate that the infection was spread from a lavatory seat. Colebrook (1946) found in freshly laundered ward linen streptococci of the same types as had been present earlier as the infecting organism of burns treated in the same hospital. Allison and Hobbs (1947) examined a blanket after laundering and isolated pathogenic staphylococci of the same types as those that had caused an epidemic of pemphigus neonatorum in the same hospital.

In a Memorandum dealing with the prevention of cross-infection the (British) Medical Research Council (1944) concluded that there are numerous possibilities of infection being spread by direct or indirect contact from sources in the patient's environment. On every ward article, whether sterilised or not, there are living bacteria. This contamination of ward articles may be due either to the settling of contaminated dust or to contagion from the hands of the staff, different secretions, excretions and discharges of the patients, *etc.* Hare (1941), in fact, recovered hemolytic streptococci from the hands of about 4 per cent of normal individuals.

In the Children's Clinic different ward articles were examined in case they might be sources in addition to ward air, and attempts were made to discover possible avenues of infection. In these samples beta hemolytic streptococci were regarded as indicator organisms, just as in the studies of air-borne bacteria.

These samples were taken with the idea of possible contagion by the hands of the nursing staff and by ward linen and baths. For this purpose samples were collected from the taps of wash-hand basins, nail-brushes and door handles in wards of different units, and from clean ward linen and baths, altogether 50 samples of each. The beta hemolytic streptococcus counts of these samples are shown in Table 16.

TABLE 16

OTHER POSSIBLE SOURCES OF INFECTION IN THE PATIENTS' ENVIRONMENT

Article sampled	Number of Samples	Number of Samples Yielding Beta Hemolytic Streptococci
1. Clean ward linen	50	0
2. Water taps of wash-hand basins in wards	50	6
3. Nail-brushes in wards (for use of staff) ..	50	14
4. Metal door handles in wards	50	0
5. Baths in wards	50	7

As evident from Table 16, the nail-brushes used by the nursing staff, as well as the water taps of the wash-hand basins of the wards, proved to be the most dangerous of the articles examined with a view to their share in the transmission of beta hemolytic streptococcal infections. Beta hemolytic streptococci were also cultured in 7 cases from the baths of the wards, but the samples from clean ward linen were always negative.

M. DISCUSSION

The object of the present investigation was to discover the importance of ward air in the etiology of nosocomial infections occurring in the Children's Clinic.

When the total bacterial count or one of the types of air bacteria is used as an index of aerial contamination, the number of bacteria present in ward air is not, of course, directly comparable to the infectiveness of air. However, it has been shown that there is a definite statistical correlation between the concentration of bacteria in air and the infections caused by these bacteria (Hamburger *et al.* 1944, Robertson *et al.* 1944, Buchbinder *et al.* 1945, *etc.*). In addition, Robertson (1943) has suggested that also air-borne infections of virus origin depend on the concentration of the infectious agent in the atmosphere. It should be mentioned that for instance Hare and Mackenzie (1946) consider the modes of spread of bacterial and virus infection essentially similar.

Although the great importance of air in the spread of cross-infections has been conclusively proved, several investigators have also stated that atmospheric pollution cannot be considered solely responsible for any infection. Thus van den Ende and Spooner (1941), who studied the bacterial content of the air in a surgical hospital, found the number of hemolytic streptococci to be so low as not to be comparable to the incidence of hemolytic streptococcal infections. According to Allison and Hobbs (1947) no respiratory infections are to be solely attributed to air-borne bacteria. He considers that endogenous infection and direct or indirect contact are essential and that infected hands play an important part in contact spread. In agreement with the above is the observation made by Loosli, in 1948, in connection with his studies of the importance of dust control for the disinfection of air, that »no

respiratory tract infection, whether of bacterial or virus etiology, and no wound or burn infection, is acquired only by the air-borne route». He says that there are many opportunities for spread of respiratory infections by other means than the air in an environment highly contaminated with infected dust. In their article on cross-infections in the Infants' Hospital in Boston, Brooks *et al.* (1942) also express their opinion that the relative importance of air-borne transmission of infection cannot be determined because adequate control of other routes of transmission is not possible.

The results of the present investigation agree fairly well with those reported by investigators abroad: the aerial contamination in the Children's Clinic is practically similar to that recorded in other hospitals.

However, the bacterial content of the Children's Clinic proved remarkably low as compared with the counts obtained in private households, in spite of the fact that, as far as is known, no infections occurred in these households at the time of sampling. It is possible that the bacteria in these households did not cause infections because there was immunity to these bacteria or because the virulence of the strains differs in domestic and in hospital environment.

Even if the bacterial content of ward air has generally been found to be very low (the total bacterial count here obtained averages 99 per 100 liters air, or about one organism per liter), all other conditions which may affect the bacterial contamination are much more uniform in hospital wards than in private households, schools, *etc.* On the basis of this, the differences in the bacterial content of different wards may be considered of significance, although they are relatively small as compared with those in the samples from other environments.

The results of samplings at different times of day show that the highest and lowest counts obtained from the same ward differed considerably. It also appeared that, as stated by previous investigators (Cruickshank 1935, van den Ende *et al.* 1940, Pulvertaft 1947, Löfström 1947, *etc.*), the bacterial content of air is usually highest after procedures which raise the dust in the ward, and that wet sweeping and dusting have not proved efficient enough in preventing the redispersal of dust-borne bacteria into the air. Even if dust control measures have resulted in a marked reduction of the

bacterial content of ward air (van den Ende *et al.* 1940, van den Ende and Thomas 1941, Robertson 1943, *etc.*), full agreement has not been reached on this subject (The Commission on Acute Respiratory Diseases 1946, Laurell 1948).

Most air-borne respiratory tract infections are considered due to hemolytic streptococci (J. Wright 1940, Allison *et al.* 1946). According to the results obtained in the present investigation, the number of hemolytic streptococci occurring especially in younger children's wards was very low: the median was 0 in both premature and infants' wards (667 liter air samples). This was the case also in all the other units in which children showing no signs of respiratory infections were nursed, with the exception of the eczematous children's unit. This would indicate that the number of dangerous carriers among the infants and children was low. It has earlier been shown in the same hospital that children with infantile eczema and prurigo Besnier (from which the patients in these wards were usually suffering) included a considerably greater number of heavy throat and nose carriers than the other children (Helve *et al.* 1950). Whether and to what extent the streptococci in the eczematous children's wards originate in their respiratory tract or their skin remains undecided.

The *Staphylococcus aureus* counts yielded by the larger air samples (667 liter) varied greatly. A possible explanation is that *Staphylococcus aureus* is usually so frequent in normal environment that its redispersal into the air depends on very many and insignifant factors. An additional cause may be that these staphylococci more commonly occur in large aggregations than for instance streptococci.

The human nasopharynx has usually been regarded as the primary source of air-borne streptococci and of staphylococci of the aureus group. Hemolytic streptococci were cultured from throat swabs of about one-third (33.6 per cent) of the staff. This very high incidence of streptococci is partly due to the fact that the swabs were taken during the winter months when streptococci are particularly frequent (Long *et al.* 1939, Peacock *et al.* 1939, van Ravensway 1944, *etc.*). The number of heavy throat carriers of streptococci and of nose carriers of beta hemolytic streptococci among the personnel was very low compared with the above figure — only 3.3 and 0.3 per cent respectively. It has been stated that

a single throat and nose culture is an unreliable index of the prevalence of beta hemolytic streptococci in the respiratory tract of healthy persons (The Commission on Acute Respiratory Diseases 1949). These throat and nose swabs of the staff were not taken with a view to obtaining any exact figures but in order to study the possible importance of such swabs in preventing the spread of streptococcal infections.

Certain modern methods of sterilising the air have proved of great value in reducing the bacterial content of ward air. Yet these methods have not nearly always been successful from the point of view of control of infections (The Commission on Acute Respiratory Diseases 1945, Laurell 1948, *etc.*) and there is still uncertainty regarding the modes of spread of even the commonest infections (The Commission on Acute Respiratory Diseases 1948, Loosli 1947); because of this, comparative studies of the bacterial content of different types of wards are still desirable with a view to the placement of wards, patients and nurses. The results of this investigation showed that the highest bacterial counts were obtained from the eczematous children's wards. The place of the Outpatient Department in the hospital may also conceivably be of importance in the spread of air-borne infections because aerial contamination is usually high there. As any activity in the wards serves to increase the bacterial pollution of the air, it would appear advantageous also in this respect to discharge convalescents as early as possible.

N. SUMMARY

1. The bacterial content of the air of the Children's Clinic in Helsinki has been studied with the aid of an air sampler of Bourdillon type modified by the author; its collection efficiency was 100 liters air per 3 minutes.

2. Air samples were collected during one year (Sept. 20, 1948, to Sept. 12, 1949). Within this period a total of 20 air samples were taken at each of 10 observation posts which, on the basis of preliminary tests, had been selected as typical.

3. Staining by Gram's method and microscopic examination were used and all visible colonies counted in 121 air samples (100 liter). It appeared that the majority of air bacteria were saprophytes; also that the bacterial content of ward air is generally high and that air bacteria are mostly of such types as remain viable in dried state and thus originate in the dust of the ward.

4. The results showed that the total bacterial count of ward air in the Children's Clinic is on an average 99 per 100 liters air. The air samples from the eczematous children's wards yielded the highest total bacterial counts: the median was 95.5 (mean 142) per 100 liters air. The lowest total bacterial counts were obtained from the premature wards where the median was 53.5 (mean 87), and from the wards housing infants with gastrointestinal infections (median 61, mean 77).

5. The beta hemolytic streptococcus was used as an index of air pollution and gentian violet as the selective medium for isolation of these bacteria. The number of beta hemolytic streptococci was constantly high in the eczematous children's wards (median 6, mean 6.7, per 667 liters air). In the air of wards in which children with no known respiratory tract infections were nursed, except for the eczematous children's wards, the number of air-borne hemolytic streptococci was small.

6. *Staphylococcus aureus* was used as the second indicator organism. These organisms were differentiated with Chapman's selective medium, 7.5 per cent salt phenol red mannitol agar. This organism was recovered in considerably greater numbers from the wards of older children than from those of young infants and children, with the exception of those housing eczematous infants, in which the *Staphylococcus aureus* counts were highest. According to the results obtained by this method the incidence of *Staphylococcus aureus* in ward air was highly variable and no correlation could be demonstrated between the number of air-borne organisms and infections due to this type.

7. The aerial contamination was highest during the autumn (mean 112 colonies per 100 liters air). This agrees with the previous observation that nosocomial respiratory tract infections are most prevalent in the Children's Clinic during the first and last few months of the year. No definite association could be observed between the relative humidity of air and its bacterial content.

8. Samples collected at different times of day showed that those daily routine procedures which raise the dust of the ward increased the bacterial content of the air most, both in wards where aerial contamination was usually low and in wards where it was usually high.

9. As regards hospital design it was noted that the place of the Outpatient Department and the kitchen affected the bacterial content of the central staircase. It seems that air currents rising from the kitchen on the ground floor carried bacteria from the Outpatient Department to the upper floors along the stair well.

10. Nose and throat swabs — 336 of each — were taken of members of the staff; of the former 0.3 per cent and of the latter 33.6 per cent were positive for hemolytic streptococci. Swabs were also taken of 336 people to discover carriers of *Staphylococcus aureus:* 11.9 per cent yielded positive nose cultures and 1.9 per cent positive throat cultures.

11. Air samples were collected in the homes of children nursed in the Children's Clinic (13 households), and the bacterial counts were considerably higher than those obtained in the Children's Clinic at the same time of the year. The median of the total bacterial counts from the Children's Clinic was 83 (mean 99) but that obtained in the private households c. 592 (mean c. 784) per 100 liters air.

The beta hemolytic streptococcus counts were respectively c. 3 (and c. 8) from the households, and 0 (and 0.7) from the Children's Clinic per 667 liters air.

12. Samplings were made in the new institution, the Children's Castle, on its completion and as its wards were filling with patients; they showed that the bacterial flora was qualitatively but little affected by the admission of patients.

13. Some ward articles in the Children's Clinic, *e.g.* nail-brushes, baths and water taps, appeared to be highly contaminated with bacteria and may thus, in addition to air, play a part in the spread of infection.

O. REFERENCES

ACKLIN, O.: Ars. med. 1946:*36:*170.

ALLISON, V. D.: Lancet 1938:*I:*1067.

ALLISON, V. D., BOURDILLON, R. B., CRAIG, W. S., CROOKS, J., CROSBIE, W. GAISFORD, W., GUNN, W., LIGHTWOOD, R., SPENCE, J. C., VINING, C. W. &. WATKINS, A. G.: Brit. M. J. 1946:*I:*673.

ALLISON, V. D. & BROWN, W. A.: J. Hyg. 1937:*37:*153.

ALLISON, V. D. & HOBBS, B. C.: Brit. M. J. 1947:*2:*1.

ALSTON, J. M.: Proc. Roy Soc. Med. 1947:*40:*383.

ANDERSON, P. H. R., BUCHANAN, J. A. & MCPARTLAND, J. J.: Brit. M. J. 1944:*I:*616.

ANDREWES, C. H. & GLOVER, R. E.: Brit. J. Exper. Path. 1941:*22:*91.

BAKER, A. H. & TWORT, C. C.: J. Hyg. 1941:*41:*117.

BOURDILLON, R. B., LIDWELL, O. M. & THOMAS, J. C.: J. Hyg. 1941:*41:*197.

BOURDILLON, R. B. & COLEBROOK, L.: Lancet 1946:*I:*561.

BRENNEMANN, J.: Am. J. Dis. Child. 1932:*43:*577.

BROOKS, G. L., WILSON, U. & BLACKFAN, K. D.: Aerobiology 1942:228.

BUCHBINDER, L.: Aerobiology 1942:267.

BUCHBINDER, L. & PHLEBS, E. B.: J. Bact. 1941:*42:*345.

BUCHBINDER, L., SOLOTOROVSKY, M. & SOLOWEY, M.: Proc. Soc. Exper. Biol. Med. 1938:*38:*570.

BUCHBINDER, L., SOLOWEY, M. & PHLEBS, E. B.: J. Bact. 1941:*42:*353.

BUCHBINDER, L., SOLOWEY, M. & SOLOTOROVSKY, M.: Am. J. Pub. Health. 1938:*28:*61.

BUCHBINDER, L., SOLOWEY, M. & SOLOTOROVSKY, M.: Ashve J. Sect. 1945:July 389.

DU BUY, H. G., HOLLAENDER, A. & LACKEY, M. D.: Pub. Health. Rep. 1945:Suppl. *184:*1.

BØE, J.: Nord. Med. 1946:*32:*2287.

CHALLINOR, I. S. W. & DUGUID, J. P.: Edinburgh. M. J. 1944:*51:*388.

CHAPMAN, G. H.: J. Bact. 1945:*50:*201.

CHAPPLE, C. C.: Aerobiology 1942:251.

CHAPPLE, C. C. & KENNEY, A. S.: Am. J. Dis. Child. 1939:*57:*1058.

COLEBROOK, L.: Brit. M. J. 1933:*2:*723.

COLEBROOK, L.: J. Obst. & Gynaec. 1946:*III:*114.

COMMISSION ON ACUTE RESPIRATORY DISEASES: Am. J. Hyg. 1946:*43:*65.

COMMISSION ON ACUTE RESPIRATORY DISEASES: Am. J. Hyg. 1946:*43:*120.

COMMISSION ON ACUTE RESPIRATORY DISEASES: Am. J. Hyg. 1949:*50:*168.
COOK, G. T. & MUNRO-ASHMAN, D.: Brit. M. J. 1949:*I:*345.
CRISTIE, W. C. & WRIGHT, H. D.: Lancet 1941:*I:*656.
CROWLEY, N., DOWNIE, A. W., FULTON, F. & WILSON, G. S.: Lancet 1941: *II:*590.
CRUICKSHANK, R.: J. Path. & Bact. 1935:*41:*367.
CRUICKSHANK, R.: Brit. M. J. 1946:*II:*831.
CRUICKSHANK, R. & GODBERG, G. E.: Lancet 1939:*I:*741.
CRUICKSHANK, R. & WRIGHT, J.: Proc. Roy. Soc. Med. 1947:*40:*381.
CRUMB, C. & WELLS, W. F.: J. Bact. 1947:*54:*277.
DEICHER, H.: Ztschr. f. Hyg. u. Infektionskr. 1928:*108:*167.
DICK, G. F., DICK, G. H. & WILLIAMS, J. L.: Am. J. Dis. Child. 1928:*35:*955.
DOLD, H. & v. ESSEN, K.: Ztschr. f. Hyg. u. Infektionskr. 1944:*125:*437.
DOWNES, A. & BLUNT, T. P.: Proc. Roy. Soc. Med. 1878:*26:*488.
DUGUID, J. P.: Brit. M. J. 1946:*I:*265.
DUGUID, J. P. & WALLACE, A. T.: Lancet 1948:*II:*845.
DUMBELL, K. R., LOVELOCK, J. E. & LOWBURY, E. J.: Lancet, 1948:*II:* 183.
DUNKLIN, E. W. & PUCK, T. T.: J. Exper. Med. 1948:*87:*87.
DZIALOSZYNSKI, I.: Ztschr. f. Kinderh. 1926:*41:*685.
VAN DEN ENDE, M., EDWARDS, D. G. & LUSH, D.: Lancet 1941:*I:*716.
VAN DEN ENDE, M., LUSH, D. & EDWARDS, D. G.: Lancet 1940:*II:*133.
VAN DEN ENDE, M. & SPOONER, E. T. G.: Lancet 1941:*I:*751.
VAN DEN ENDE, M. & THOMAS, J. C.: Lancet 1941:*2:*755.
ERNST, J.: Disput. Kjöbenhavn 1942, The Epidemiological Significance of Grouping and Typing the Haemolytic Streptococci.
EVANS, J. B.: J. Bact. 1948:*55:*793.
FINGERLAND, A.: Zentralbl. f. Chir. 1942:*69:*1632.
FINLAND, M.: Aerobiology 1942:212.
FISHER, G.: Hyg. Revy 1949:*38:*1.
FLÜGGE, C.: Ztschr. f. Hyg. u. Infektionskr. 1897:*25:*179.
FRISH, A.: J. Infect. Dis. 1938:*62:*40.
GARROD, L. P.: St. Bart's Hosp. med. Rep. 1933:*66:*203; quoted by WILSON, G. S. & MILES, A. A.: Topley et Wilson's Principles of Bacteriology and Immunity. 3 Ed. London 1946:135.
GARROD, L. P.: J. Infect. Dis. 1935:*57:*247.
GARROD, L. P.: Brit. M. J. 1944:*I:*245.
GAUSTAD, V.: Nord. Hyg. Tidskr. 1945:*26:*47.
GEDDA, E.: Nord. Hyg. Tidskr. 1949:*3:*71.
GILLESPIE, E. H., DEVENISH, E. A. & COWAN, S. T.: Lancet 1939:*II:*870.
GLASS, V.: Lancet 1941:*I:*542.
GREENE D., BARENBERG, L. H. & GREENBERG, B.: Am. J. Dis. Child. 1941:*61:*273.
GRIFFITH, F.: J. Hyg. 1934:*34:*542.
GORDON, M. H.: Med. Off. 1903—1904:*32:*421; quoted by BUCHBINER, L. *et al.:* Ashve J. Sect. 1945:*July:*389.
HAMBURGER, M. Jr.: J. Infect. Dis. 1944:*75:*58.

Hamburger, M. Jr. & Green, M. J.: J. Infect. Dis. 1946:*79:*33.
Hamburger, M. Jr., Green, M. J. & Hamburger, V. G.: J. Infect. Dis. 1945:*77:*68.
Hamburger, M. Jr., Green, M. J. & Hamburger, V. G.: J. Infect. Dis. 1945:*77:*96.
Hamburger, M. Jr. & Lemon, H. M.: J.A.M.A. 1946:*130:*836.
Hamburger, M. Jr., Lemon, H. M. & Platzer, R. F.: Am. J. Hyg. 1949:*49:*140.
Hamburger, M. Jr., Puck, T. T., Hamburger, V. G. & Johnsson, M. A.: J. Infect. Dis. 1944:*75:*79.
Hamburger, M. Jr. & Puck, T. T. & Robertson, O. H.: J. Infect. Dis. 1945:*76:*208.
Hamilton, A.: J.A.M.A. 1905:*44:*1108.
Hare, R.: J. Path. & Bact. 1935:*41:*499.
Hare, R.: Lancet 1940:*I:*109.
Hare, R.: Lancet 1941:*I:*85.
Hare, R.: Proc. Roy. Soc. Med.: 1947:*40:*383.
Hare, R. & Mackenzie, D. M.: Brit. M. J. 1946:*I:*865.
Hare, R. & Willits, R. E.: Canad. M. A. J. 1941:*44:*230; quoted by Robertson, O. H.: Science 1943:*97:*495.
Harris, T. N. & Stokes, J., Jr.: Am. J. Med. Sc. 1945:*209:*152.
Hart, D.: Aerobiology 1942:186.
Hart, D. & Scheibel, H. M.: Arch. Surg. 1939:*38:*787.
Hart, D. & Upchurch, S. E.: Ann. Surg. 1942; quoted by Hart, D.: Aerobiology 1942:186.
Hartmann, O.: Nord. Med. tidskr. 1938:*15:*641.
Heise, R.: Arb. aus dem Kais. Gesundh. Amt. Berlin 1909:*30:*93.
Helve, A.: Duodecim 1948:499.
Helve, A., Pätiälä, R. & Oker-Blom, N.: Acta Paediat. 1950, in Press.
Hettche, H. O. & Schwab, A.: Arch. f. Hyg. 1940:*123:*283.
Heymann, B.: Ztschr. f. Hyg. u. Infektionskr. 1899:*30:*139.
Hirschfeld, J. W.: Surg., Gynec. & Obst.: 1941:*73:*73.
Hirvisalo, K. F.: Acta Soc. Med. Fenn. Duodecim 1933: Ser. A, *Tom XVI, Sect 12: 1.*
Horewood, M. P.: J. Bact. 1931:*21:*14.
Jennison, M. W.: Aerobiology 1942:106.
Kalbak, K.: Disput. København 1942, Experimentelle og Kliniske Undersøgelser over O-Streptolysin og Forekomsten af O-Antistreptolysin i Serum.
Keevil, N. L. & Camps, F. E.: Lancet 1937:*II:*207.
Kenny, M. & Barber, M.: Brit. M. J. 1944:*1:*809.
McKhann, C. F., Steeger, A. & Long, A. P.: Am. J. Dis. Child. 1938: *55:*579.
Kirstein, F.: Ztschr. f. Hyg. u. Infektionskr. 1900:*35:*123.
Koch, R.: Mitteilungen aus dem Kaiserlichen Gesundheitsamte 1884:*2:*1.
Kokko, U. P.: Acta path. et microbiol. Scandinav. 1946:*23:*528.
Lagercrantz, R.: Nord. Med. 1948:*40:*2143.

Lancefield, R.: J. Exper. Med. 1933:*57:*91.
Lange, B.: Erg. d. Hyg. Bakt. Immunitätsforsh. u. exp. Therap. 1928:*9:* 237.
Larsson, Y.: Acta Paediat. 1945:*32:*539.
Laurell, G.: Acta Paediat. 1948:*35:*182.
Laurell, G., Löfström, G., Magnusson, J. H. & Ouchterlony, Ö.: Acta Med. Scandinav. 1947:*Suppl. 196:*602.
Laurell, G., Löfström, G., Magnusson, J. H. & Ouchterlony, Ö.: Acta Med. Scandinav. 1949:*134:*189.
Laschtschenko: Ztschr. f. Hyg. u. Infektionskr. 1899:*30:*125.
Lester, W., Robertson, O. H., Puck, T. T. & Wise, H.: Am. J. Hyg. 1949:*50:*175.
Lichtenstein, A.: Acta Paediat. 1932:*12:*95.
Lichtenstein, A.: Acta Paediat. 1935:*17 suppl. I:*36.
Lojander, W.: Acta Soc. Med. Fenn. Duodecim 1934: Ser. A, *Tom. XVI, sect 5: 1.*
Long, A., McKhann, C. F. & Cheney, L. L.: Am. J. Dis. Child. 1939:*57:* 1363.
Long, A., McKhann, C. F. & Cheney, L. L.: Am. J. Dis. Child. 1940:*60:*322.
Loosli, C. G.: Am. J. Pub. Health 1947:*37:*353.
Loosli, C. G.: Am. J. Pub. Health 1948:*38:*409.
Loosli, C. G., Appel, E., Robertson, O. H. & Lemon, H. M.: Proc. Soc. Exper. Biol. & Med. 1943:*53:*205.
Loosli, C. G., Robertson, O. H. & Puck, T. T.: J. Infect. Dis. 1943:*72:*142.
Loosli, C. G. & Smith, M. D. H.: Am. J. Dis. Child. 1949:*77:*122.
Luckiesh, M., Holloday, L. L. & Taylor, A. H.: Gen. Elect. Rev. 1946: *49:*8.
Luckiesh, M. & Taylor, A. H.: Mag. of Light 1946:*2:*303.
Luckiesh, M. & Taylor, A. H.: Ashve J. Sect. 1947:*January:*113.
Luckiesh, M., Taylor, A. H. & Holloday, L. L.: J. Bact. 1946:*52:*55.
Löfström, G.: Nord.. Med. 1947:*36:*2565.
MacDonald, K.: Am. J. Hyg. 1940:*31:*74.
McFarlan, A. M.: Brit. M. J. 1938:*2:*939.
Medical Research Council, War Memorandum 1944:No 11.
Meyer, L. F.: Ztschr. f. Kinderh. 1913:*7:*233.
Miles, A. A.: Brit. M. J. 1940:*II:*855.
Mitman, M.: Brit. M. J. 1945:*I:*71.
Moulton, S., Puck, T. T. & Lemon, H. M.: Science 1943:*97:*51.
Muhl, G.: Nord. Med. 1948:*38:*851.
Del Mundo, F. & McKhann, C. F.: Am. J. Dis. Child. 1941:*61:*213.
Neisser, M.: Ztschr. f. Hyg. u. Infektionskr. 1898:*27:*175.
Neufeld, F.: Am. Rev. Tuberc. 1927:*609:*15.
Nyman, H. O.: Nord. Hyg. Tdskr.: 1947:*3:*93.
Okell, C. C. & Elliott, S. D.: Lancet 1936:*II:*836.
Packalén, T.: Acta path. et microbiol. Scandinav. 1949:*26:*586.
Packalén, T. & Bergqvist, S.: Acta Med. Scandinav. 1947:*127:*291.
Parker, M. T. & Kennedy, J.: J. Hyg. 1949:*47:*213.

PEACOCK, S., BIGGLER, J. A. & WERNER, M.: Am. J. Dis. Child. 1939:*57:*759.
PETERS, S.: Med. Officer 1946:*75:*9; quoted by Nord. Med. 1946:*31:*1933.
PHLEBS, E. B. & BUCHBINDER, L.: J. Bact. 1941:*42:*321.
PIKE, R. M. & FASHENA, G. J.: Am. J. Pub. Health. 1946:*36:*611.
PINCUS, S. & STERN, A. C.: Am. J. Pub. Health. 1937:*27:*321; quoted by FRIDERICIA, L. S.: Nord. Med. 1945:*28:*2177.
PUCK, T. T., ROBERTSON, O. H. & LEMON, H. M.: J. Exper. Med. 1943:*78:* 387.
PULVERTAFT, R. J. V.: Brit. M. J. 1947:*II:*517.
RATCLIFFE, H. L.: J. Bact. 1946:*52:*259.
RAVENSWAY, A. C. VAN: J.A.M.A. 1944:*126:*486.
RECENT STUDIES ON DISINFECTION OF AIR IN MILITARY ESTABLISHMENTS: Am. J. Pub. Health. 1947:*37:*189.
ROBERTSON, C. E., DOYLE, M. E., TISDALL, F. F., KOLLER, L. R. & WARD, F. S.: Am. J. Dis. Child. 1939:*58:*1023.
ROBERTSON, C. E., DOYLE, M. E. & TISDALL, F. F.: J.A.M.A. 1943:*121:*908.
ROBERTSON, O. H.: Science, 1943:*97:*495.
ROBERTSON, O. H.: Harvey Lect. Series 1943:*38:*227.
ROBERTSON, O. H. & BIGG, E., MILLER, B. F. & BAKER, Z.: Science 1941: *93:*213.
ROBERTSON, O. H. & HAMBURGER, M. JR., LOOSLI, C. G., PUCK, T. T., LEMON, H. M. & WISE, H.: J.A.M.A. 1944:*126:*993.
ROBERTSON, O. H., PUCK, T. T. & WISE, H.: J. Exper. Med. 1946: *84:*559.
ROSENSTERN, I.: Aerobiology 1942:242.
SAUER, L. W., MINSK, L. D. & ROSENSTERN, I.: J.A.M.A. 1942:*118:*1271.
SCHNEITER, R., DUNN, J. E. & CAMINITA, B. H.: Pub. Health. Rep. 1945: *60:*789.
SCHWENTKER, F. F., JANNEY, J. H., GORDON, J. E.: Am. J. Hyg. 1943:*38:* 27.
STILLMAN, E. G. & BRANCH, A.: J. Exper. Med.: 1925:*41:*623.
THOMAS, J. C. & VAN DEN ENDE, M.: Brit. M. J. 1941:*I:*953.
TRILLAT, A.: Bull. Acad. de Méd. Paris: 1938:*119:*64.
TRILLAT, A. & KANEKO, R.: Compt. rend. Acad. d. sc. USRR 1921:*173:*109; quoted by ROBERTSON, O. H.: Science 1943:*97:*495.
TORREY, J. & LAKE, S.: J.A.M.A. 1941:*117:*17.
WALTER, W. G. & HUCKER, G. J.: J. Infect. Dis. 1942:*71:*237.
WATKINS, A. G. & LEWIS-FANING, E.: Brit. M. J. 1948:*I:*616.
WELLS, M. W.: Am. J. Hyg. 1944:*40:*279.
WELLS, M. W.: J.A.M.A. 1945:*129:*179.
WELLS, W. F.: Am. J. Pub. Health. 1933:*23:*58.
WELLS, W. F.: Am. J. Hyg. 1934:*20:*611.
WELLS, W. F.: J. Indust. Hyg. & Toxicol. 1935:*17:*253.
WELLS, W. F.: Proc. of the Third International Congress for Microbiology 1939; quoted by WELLS, W. F. et WELLS, M. W.: Aerobiology 1942:99.
WELLS, W. F.: Science 1940:*91:*172.
WELLS, W. F. & HENLE, W.: Proc. Soc. Exper. Biol. & Med. 1941:*48:*298.

Wells, W. F. & Lurie, M.: Am. J. Hyg. 1941:*34:*21.
Wells, W. F. & Ratcliffe, H. L. & Crumb, C.: Am. J. Hyg. 1948:*47:*11.
Wells, W. F. & Riley, E. C.: J. Indust. Hyg. & Toxicol. 1937:*19:*513.
Wells, W. F. & Stone, W. R.: Am. J. Hyg. 1934:*20:*619.
Wells, W. F. & Wells, M. W.: J.A.M.A. 1936:*107:*1698.
Wells, W. F. & Wells, M. W.: Am. J. Pub. Health. 1938:*28:*343.
Wells, W. F. & Wells, M. W.: Aerobiology 1942:99.
Wells, W. F., Wells, M. W. & Mudd, S.: Am. J. Pub. Health. 1939:*29:*863.
Wells, W. F., Winslow, C-E. A. & Robertson, E. C.: Am. J. Pub. Health. 1946:*36:*324.
Wheeler, S. M. & Duckett Jones, T.: Aerobiology 1942:237.
Wheeler, S. M., Foley, G. E. & Duckett Jones, T.: Science 1941:*94:*445.
Wheeler, S. M., Ingraham, H. S. & Hollaender, A.: Am. J. Pub. Health. 1945:*35:*457.
White, E.: Lancet 1936:*I:*941.
Williams, R. E. O.: J. Path. & Bact. 1946:*58:*259.
Winslow, C-E. A. & Kligler, I. J.: Am. J. Pub. Health. 1912:*2:*663; quoted by Laurell, G. et al.: Acta Med. Scandinav. 1947:*196:*602.
Wohlfeil, T.: Zschr. f. Hyg. u. Infektionskr. 1937:*119:*667.
Wright, H. D., Shone, H. R. & Tucker, J. R.: J. Path. & Bact. 1941: *51:*111.
Wright, J.: J. Hyg. 1940:*40:*647.
Wright, J., Cruickshank, R. & Gunn, W.: Brit. M. J. 1944:*I:*611.
Ylppö, A.: Acta Paediat. 1935:*17*, Suppl. I:107.
Ylppö, A.: Duodecim 1948:168.